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THE STRUCTURE OF THE PLACENTAL COLUMN IN THE GENUS *MELANDRIUM* (CARYOPHYLLACEAE)

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Introduction

It is generally admitted that the placentation in the Caryophyllaceae is only free-central in appearance. On the contrary, the early stages of development of the ovary and the presence of septa, persistent to a varying degree according to the genus, go to prove that the placentation is really axile in an ovary of plurilocular origin.

There are many observations which give weight to this point of view. For example, Lister in her paper on morphogenesis (1884) reveals the carpellary origin and appendicular nature of the placentae in various Caryophyllaceae. Not only in the Silenoideae, but also in the Paronychioideae and the Alsinoideae where the structure of gynoecium is more difficult to interpret owing to the degree of reduction it has incurred. The growing point of the floral axis enters but little into the structure of the gynoecium, and that only during the initial stages of its formation, as was noted by Schaefer (1890): "... habe ich ein ausgiebigeres Längenwachstum seit Anlage der Karpellhöcker nicht bemerken können." Apical growth is halted and replaced by that of the lateral appendages (a process which recalls the development of the inflorescence, a dichasial cyme). As a result of this mode of development, the carpels arise as lateral outgrowths but finally occupy an apparently terminal position when adult.

However, various authors have observed the presence of a vascular strand in the middle of the central column in the ovary of certain species:

1. *Melandrium diurnum* (Sibth.) Fries, observations by van Tieghem, sub *Lychnis dioica* L.; also by Lister (1884) and Thomson (1942).

2. *Melandrium album* (Miller) Garcke, Dickson (1936) and Thomson (1942).

3. *Cerastium vulgatum* L., Thomson.

Of the four authors cited only Thomson and van Tieghem express an opinion on the nature and significance of this central strand. They considered it to be a prolongation of the floral axis. Neither author indicates where these strands end. Van Tieghem (1871), however, gave a fairly accurate, though schematic, account of its mode of development. He also observed a displacement of the vascular supply at the level of the uppermost ovules.

Materials and Methods

Flowers were cleared in chloral-lactophenol (*see* Vautier, 1949). It is easier to follow the course of the vascular bundles, stained with gentian violet, in a transparent medium, than to reconstruct their path mentally from the study of a series of sections. A binocular dissecting microscope offered sufficient magnification for easy observation of all but the finest strands in the ovary. These were studied under a phase-contrast microscope using magnifications up to 80 diameters, the ovary being suitably dissected out beforehand.

Observations were carried out on very favourable fresh material cultivated in the botanical gardens of Geneva in a species of *Melandrium* sect. *Gastrolychnis* collected by A. Zimmermann in Nepal. The seed was obtained from the collection of Zimmermann 1527[1].

1. Zimmermann 1527 is considered to represent a new species a description of which will be published shortly. Zimmermann 1425 and 1716 are further herbarium specimens of the same species.

Species of the section *Gastrolychnis* have very broad hermaphrodite flowers and are thus suitable material for the study of the placental column. Unfortunately they are delicate plants and are not easily cultivated in the Geneva climate. Fresh material of *Heliosperma*, *Dianthus*, *Saponaria* and *Silene* obtained from the botanical gardens of Geneva, was also examined, but, generally speaking, owing to the narrowness of the ovary or to a reduction in the number of carpels it was far less favourable for the study of the placental column. It revealed but traces of the features under consideration and hence only partial confirmation.

Drawings and observations are therefore based essentially on *Melandrium*.

Observations

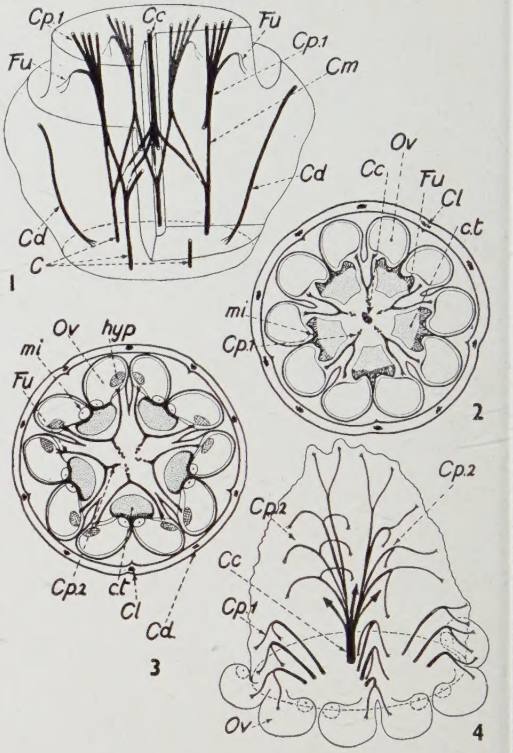
Figure 1 is a reconstruction of the vascular supply of the base of the ovary of *Melandrium* as visible in a cleared and dissected specimen. In order to simplify the drawing, the ovules have been omitted and only two of the five dorsal strands (*Cd*) of the carpels without their laterals (*Cl*) have been included. A slice has been cut out of the front right-hand portion of the organ.

The drawing shows that at the level of the base of the carpels, the vascular system is composed as follows:

1. Innermost, five epipetalous strands (*C*) which will supply the placentae.
2. Outermost, five episepalous strands (*Cd*) which form the dorsal strands of the five carpels. Their origin has no visible connection with that of the *C* strands.

A little higher but still below the base of the ovarian cavity the *C* strands divide to form the carpellary marginals (*Cm*), which join laterally to form the placental bundles (*Cp*) opposite to the corresponding dorsal strand (*Cd*).

The placental bundles give off numerous branches, all directed towards the surface of the placental column where they give rise to funicular strands (*Fu*). This branching takes place in such a fashion as to give a laminated appearance to each vascular bundle, the laminations being radially disposed relative to the axis of the column.



FIGS. 1-4 — *Melandrium* (*C* & *Cm*, marginal carpellary strands; *Cc*, central cord of the placental column; *Cd*, dorsal strands of the carpels; *Cl*, lateral strands of the carpels; *Cp1*, placental strands of the carpels in lower part of the ovary; *Cp2*, placental strands of the carpels in upper part of the ovary; *c.t*, pollen tube conductive tissue; *Fu*, funicular strand; *hyp*, hypostase; *mi*, micropyle; *Ov*, ovule). Fig. 1. Dissected base of the ovary; the placental column is in the centre and the base of the outer wall of the ovary is on the outside. Fig. 2. Transverse section through the bottom third of the ovary. Fig. 3. Transverse section through the top third of the ovary. Fig. 4. Schematic reconstruction of the vascular supply to the top of the placental column. The ovary at anthesis is 7·5 mm high and 4·0 mm in diameter. For details *see* text.

In the axil of each fork formed by the separating *Cm* strands a small ill-defined strand passes to the centre of the column to unite with the four others forming a central cord (*Cc*) with internal xylem but with an irregular cross-section.

Figure 2 is a transverse section of the ovary about one-third from its base. The central cord (*Cc*), the radiating placental strands (*Cp1*), the funicular strands

(*Fu*), and the ovules are clearly visible. The micropyles of the latter are embedded in the conductive tissue formed by the residue of the septa. The dorsal (*Cd*) and lateral (*Cl*) strands of the carpels are visible in the ovary wall.

Figure 3 is a transverse section of the same ovary but in the region of the upper part of the placental column. At this level the upper placental bundles (*Cp2*) alternate with the dorsal strands and the bundles.

A study of the placental column between the levels of the sections represented in Figs. 2 and 3 reveals a connection between the *Cp2* strands and the central cord *Cc*. In fact, at the level of the eighth or ninth pair of ovules the *Cp1* produce no more funicular strands but the central bundle forms five spreading branches each on the radius of a pad of conductive tissue. These *Cp2* strands provide the funicular strands to the three or four remaining pairs of ovules. The ovules retain their normal position, only their funicles are directed towards the septal residues and not as below to a position facing the dorsal carpellary strand. Figure 4 is a schematic illustration of this layout. In order to simplify the drawing only three of the *Cp1* bundles, two of the *Cp2*, and the lowest row of ovules are illustrated.

Discussion

1. The presence of a central vascular cord (*Cc*) in the placental column of the broad-flowered species of *Melandrium* confirms the observations of van Tieghem (1871, 1898), and Thomson (1942). Traces of this cord are also occasionally found in other, more narrow-flowered, Silenoideae.

2. The cord (*Cc*) originates from the same source as the placental strands (*Cp1*); they arise as five traces from the axils of the marginal carpellary strands. This again confirms van Tieghem's observations. However, this author considers the *Cc* cord to be perfectly symmetrical. This has not been found to be the case and, on the contrary, it appears fairly irregular in cross-section. In the *Cc* cord the xylem is central, surrounded by the phloem, just as in the *Cp1* strands.

3. The uppermost pairs of ovules alternate with the lower pairs and are attached to placental strands *Cp2* which face the septa and not, as do the *Cp1* strands, the dorsal carpellary strands (*Cd*). Van Tieghem (1871) also noted this alternation. He explained that this was caused by a branching of the *Cp1* strands and of a subsequent redistribution of the branches in the upper region of the ovary (*Cp2*).

It has been seen that on this point van Tieghem was mistaken; no such redistribution takes place, and the *Cp1* strands end below the top of the placental column.

4. It has been clearly established that the *Cp2* strands are derived from the cord *Cc* which functions as composite placental cord. It is clear that the presence of a central vascular strand, on the one hand, and displacement of the upper pairs of ovules, on the other, are two aspects of the same problem; a problem which assumes a considerable importance when dealing with the floral organization of the Silenoideae.

Three hypotheses may give possible solution to this problem.

1. According to van Tieghem and Thomson, and by analogy with the case of *Aquilegia canadensis* demonstrated by Eames (1931), this central cord would be an extension of the floral axis. Such would be the simplest hypothesis but would provide no explanation of the alternating position of the uppermost pairs of ovules. Nor does it take into account the placental function of the cord *Cc*. Finally it is strange that a bundle of such importance should be considered to be but a nonfunctional relic of the floral axis!

2. Two whorls of carpels would be represented, the upper whorl being rudimentary and limited to a few pairs of ovules. This hypothesis is of a purely academical nature as there are no morphological features to substantiate it.

3. The central cord *Cc* is composed of a reserve portion of the placental strands destined to the irrigation of the uppermost ovules. This last hypothesis accounts for both the origin and the function of the central cord. It does not, however, explain why the upper ovules are differently

placed from the rest and why they are independently vascularized.

The morphogenesis of the flower supplies a clue to that part of the problem. Lister (1884) showed that in *Melandrium diurnum* (Sibth.) Fries, the carpels originate in a lateral position just below the growing point as two to five small humps, according to the species being considered. These humps develop in a lateral direction, that is, parallel to the circumference of the axis until they meet and fuse to form a continuous ridge around the apex. From the points of fusion of these humps there is centripetal growth to form the partition walls separating the loculi of each carpel (five in *Melandrium diurnum*). The growth of the carpellary tissue soon covers the entire apex whose own growth at this point ceases completely. From then on the carpels are in a terminal position and it is difficult to consider the central column, and therefore the cord *Cc* to be other than carpellary in origin. The young ovary is thus made up of two to five open pockets. It elongates in a special manner, the outer walls growing much more rapidly than the septa or the placental column. A central depression is thus formed and the ovary at this stage can be compared to a group of stalls. The base of the ovary is plurilocular whilst the upper portion is incompletely divided by partition walls, the inner edges of which are free. The outer walls finally close over the top of the ovary without, however, any complete partitioning of the cavity in the upper part. A capsule is thus formed which is two to five locular at the base and has a corresponding number of half closed " bays " at the top. Before anthesis the central portion of the septa is resorbed to a varying degree according to the species. In a broad-flowered *Melandrium* only a few traces of the septa remain against the outer walls of the ovary, whilst they persist against the central column between each double row of ovules as a pad of the spongy conductive tissue into which the ovules immerse their micropyles. As for the free edges of the septa, they prolong these pads of spongy tissue from the top of the placental column to the roof of the ovarian cavity forming thus two to five bands of tissue alternating, in position, with that of the styles. These bands disintegrate after anthesis.

In the ovary the ovules appear progressively by pairs from a very early stage, even before the closing of the ovarian cavity. The order in which these ovules develop is not quite that in which it might be expected to occur. The first ovules are formed on the free edges of the septa in the upper half of the ovary and progressively towards the top: their placentation is parietal, but there are few of them. The majority appear later in the lower half of the ovary, as a double vertical row in the inner angle of each carpel: their placentation is axile (Lister, 1884).

It follows, therefore, that an ovule may occupy two different positions according as to whether it has developed against the placental column or on the free edge of a septum. In the former they are facing the dorsal carpellary strand in the lower half of the ovary (the pair of ovules here belong to the fused opposite margins of the same carpel) whilst in the latter, they are parallel to the edges of the carpels (the two ovules of each pair here belong to the fused margins of neighbouring carpels).

It is not unexpected, in view of the independent fashion in which the two sets of ovules arise, that they should be vascularized from largely independent sources. It is also not surprising that in the narrow-flowered species, where the ovules of the " upper system " are but few in number, the vascular development should be so slight as to be hardly noticeable and hence easily overlooked.

The mode of development of the ovary as described by Lister thus fully substantiates the hypothesis that the central strand is of carpellary origin, and does in fact represent a supplementary placental system which, owing to the displacement and regrouping of the upper ovules has become " condensed " into a spurious central axis in the lower zone of the ovary. Phylogenetically speaking the *Cc* cord does not, therefore, represent a relic of the central axis but rather a portion of a bivalent placental system characteristic of the entire Silenoideae. This concept, based on the third hypothesis listed, is considered to be the most satisfactory.

Summary

Several authors have noted the presence of a central vascular strand in the placental column of certain Caryophyllaceae, especially in the case of the genus *Melandrium*.

Van Tieghem and Lister interpreted this structure as a relic of the floral axis. However, after a study of the material these strands have been seen to form a part of the placental vascular supply. Moreover, this column supplies the traces of the upper ovules, which are displaced relative to their lower neighbours. They are in effect attached to the margin of the septa in the upper portion of the ovary, where the latter are not fused to each other.

The central strand appears, from its source, structure and especially from destination, to be nothing more than a portion of the placental vascular system supplying the uppermost ovules, since, owing to their position, these cannot be normally vascularized. This central column is, therefore, not a relic of the axis. Further, the placental column is, in spite of appearances, almost entirely of carpellary origin.

I wish to offer my most sincere thanks to Mr Marius Noul, vice-president of the council of the city of Geneva and delegate in charge of Museums, and to Professor Baehni, director of the Conservatoire Botanique, for having conferred on me the honour of the curatorship of the Boissier collections, which has enabled me to carry out this work. I wish also to express my warmest gratitude to Dr C. E. B. Bonner, Curator at the Conservatoire Botanique, Keeper of the Stephani, Müller-argoviensis and Fuckel collections, for his constant encouragement, his ever ready advice and criticism and for the time and labour involved in writing an English text for this work.

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THE CAMPYLOTROPOUS OVULE

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Introduction

Ovules are classically divided into three schematic types:

1. The orthotropous or atropous ovule (e.g. Juglandaceae, Polygonaceae) where the axis of the nucellus is an extension of that of the funicle.

2. The anatropous ovule (the most frequent type), wherein the nucellus has become " reversed " owing to a bending of the funicle below the chalaza, the micropyle becoming adnate to the funicle.

3. Finally, the campylotropous ovule where the proximity of the micropyle to the funicle is due to a bending in the region of the nucellus.

This classification is not sufficient to cover the great diversity of forms of ovules. In fact, Warming (1913) introduced two more terms, hemi-anatropous and hemi-campylotropous, to cover intermediate forms. But, he gave no clear definition of his terms. A few years later, Goebel (1933) concluded that only the categories referred to as atropous and anatropous correspond to well defined structures. He proposed the term hemitropous to cover those types where the nucellus is not bent and only turned through about 90° (" nur um 90° gedreht und annähernd quer zum Funiculus ausgewachsen "). This state is to be found, for example, in some Primulaceae and Scrophulariaceae such as *Glaux maritima* and *Torenia asiatica* (Goebel, 1933). He restricted the use of the term campylotropous to ovules wherein the nucellus is bent on one side only (" einseitig gekrümmt ") as in the Leguminosae. In cases where the curvature of the nucellus is more accentuated and affects both dorsal and ventral sides, he uses the term amphitropous (zweiseitig gekrümmt). Such an ovule is folded about its centre to form a hog-back. The pea, *Pisum sativum* L., is a good example of this type of ovule (*see* Pitot, 1936). Other examples are to be found in the Cruciferae like *Capsella bursa-pastoris* (L.) Medikus and some Chenopodiaceae, such as *Atriplex hortensis* L. (Goebel, 1933).

In the amphitropous ovule the bending of the nucellus is accompanied by the formation, beneath the ventral face, of a mass of cells. This parenchymatous tissue extends, like a finger, into the arch of the nucellus. Such is the case in the Cruciferae (e.g. *Capsella bursa-pastoris*). This tissue may even penetrate the nucellus forming a perisperm which eventually becomes surrounded by the developing embryo (e.g. *Atriplex hortensis*). Goebel (1933) states that this organ, which we will call the basal body, originates either from the tissues of the funiculus and the integuments or from the nucellus itself. It supplies food reserves and subsists only for a variable span of time.

Goebel also noted that in campylotropous ovules there is an initial bending of the funicle as for an anatropous condition, but this change is never completed. They can thus be considered as modified anatropous ovules. The early stages of development in the bean, *Vicia faba* L., are very similar to those of an anatropous or a hemitropous ovule.

Goebel's nomenclature is much closer to the mark as regards the real structure of the ovule, but it is not sufficient to cover all the differences or affinities between two groups: it enables one, for example, to establish a distinction between the ovule of a bean (campylotropous) and that of a pea (amphitropous), but is of no avail for providing a distinction between the ovules of the Caryophyllaceae and most Leguminosae, all of which are considered to be campylotropous.

In order to obtain a precise classification it has been found necessary to take two further features into account: the position of the vascular strand in the funicle and of the initial stages of development of the ovule. It then becomes possible to make more useful comparisons.

Material and Methods

The following specimens were collected during 1957 in the botanical garden of Geneva:

Biscutella cichoriifolia Loisel. No. 80, *Cerastium arvense* L. No. 17, *Heliosperma quadridentatum* (Murray) Schinz & Thellg. No. 10, *Paronychia serpyllifolia* (Chaix) DC. No. 20.

It is difficult to follow accurately the course of vascular strands in an organ when using classical techniques (e.g. serial sections), and this is particularly true in the case of an organ like a funicle where the lignification of the xylem is frequently tardy. Such is also the case for the early stages of the flower bud where the vascular tissue is represented only by elongated cells in the process of differentiation.

It was preferred to clear flowers in toto using the chloral-lacto-phenol technique (*see* Vautier, 1949). A single inflorescence will furnish all stages of ovule development.

The ovaries, when they had been suitably dissected, were examined under a phase-contrast microscope.

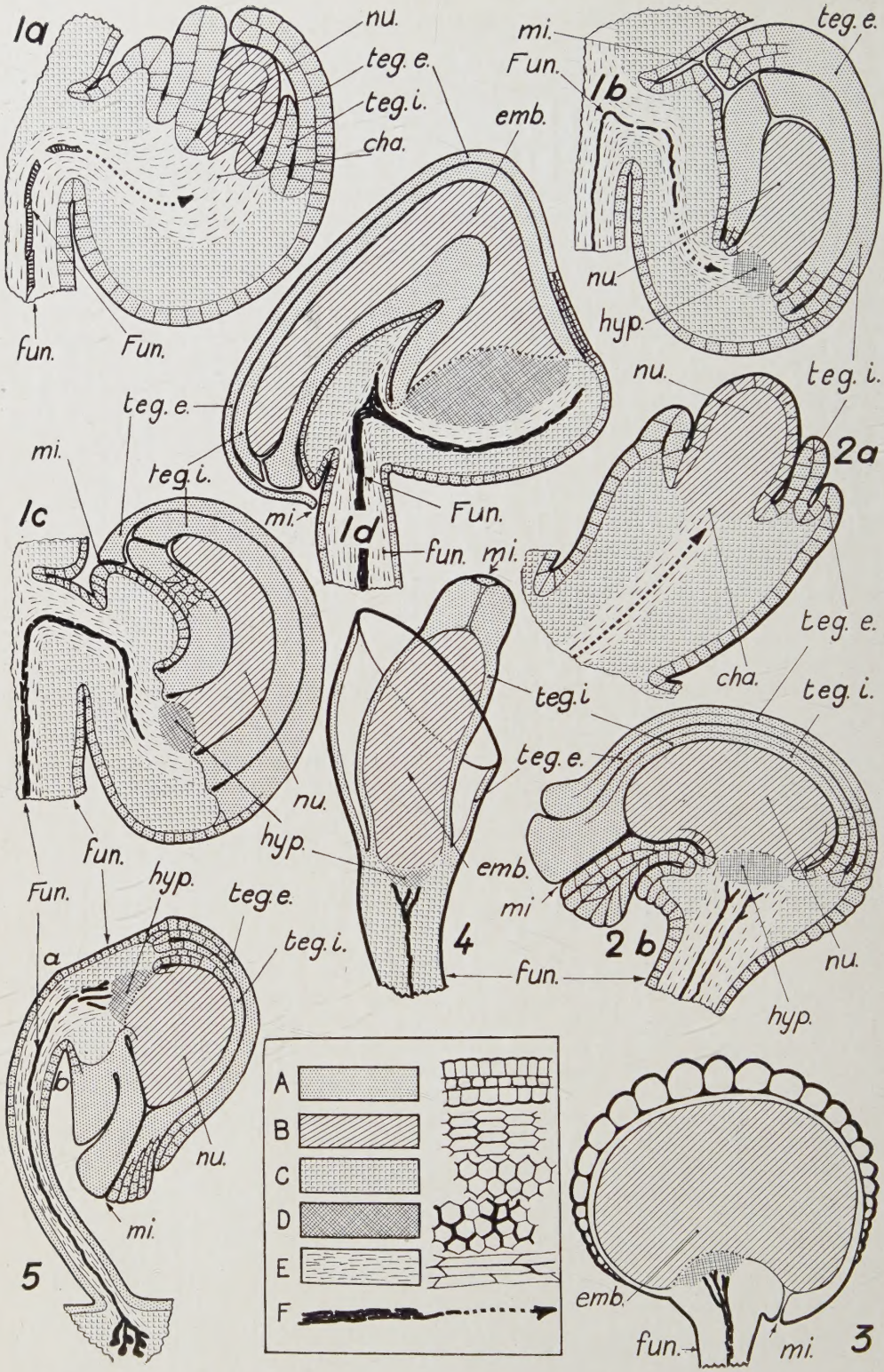
Results

An abundant selection of campylotropous ovules belonging to the three families, Caryophyllaceae, Cruciferae and Leguminosae, was studied. In the latter two, it was frequently observed that, as Goebel (1933) had pointed out, an initial bending of the funicle takes place just beneath the chalaza and that this early change is soon supplemented by a curving of the nucellus. The effect of this second distortion is to bring the micropyle into the neighbourhood of the funicle. This phenomenon is caused by the more rapid growth of the integuments on the side away from the raphis. In this way the ovule becomes campylotropous shortly before anthesis. These characteristics are further accentuated after fertilization and during seed formation.

Such an ovule could be described as being anatropous with a curved nucellus or ana-campylotropous. In fact, the axis of the nucellus (that of the basal portion at least) is parallel to that of the funicle and the chalaza has pivoted with respect to the latter as in a typical anatropous ovule. There are many good examples of this type of ovule in the Leguminosae. Pitot (1936) lists many interesting cases as *Anagyris foetida* L., *Cicer arietinum* L. and *Phaseolus vulgaris* L. In *Cercis siliquastrum* L., where the ovule is usually considered to be anatropous, the bending of the nucellus is very slight but nevertheless quite visible. The greater the delay in the curvature of the nucellus, the less marked is the final campylotropous state of the ovule. In the Caesalpinioideae the bending affects little more than the micropylar region.

In *Pisum sativum* L. the ovule develops in a similar fashion to an ana-campylotropous stage. The tissues of the raphis and of the adjacent outer integument then proliferate, forming a basal body which lifts the floor of the nucellus. This process is accentuated during seed development until finally the embryo is folded in half and the seed has developed a prominent back. As for the funicle, it is median. We have thus an amphitropous ovule derived directly from an ana-campylotropous stage. This ovule, which can be termed ana-amphitropous, bears a strong resemblance to that of other Papilionaceae. In spite of its different external shape, the basic morphological differences between the ovule of *Vicia* and that of *Pisum* are relatively unimportant. Amphitropy is caused by a third curvature which accentuates the funicular bend and extends the campylotropous distortion of the nucellus. It is due to a belated resumption of unilateral growth of the nucellus and its integuments.

An analogous condition is found in the ovule of *Biscutella cichoriifolia* Loisel. (Cruciferae). At a very early stage the future ovule is already anatropous with a bent funicle (Fig. 1a), but the nucellus is still straight. The curving of the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FIGS. 1-5.

nucellus then sets in and the ovule becomes typically campylotropous (Fig. 1b, 1c). Finally, as soon as the flower reaches maturity, the third distortion occurs and the ovule becomes ana-amphitropous (Fig. 1d). This is a most frequent type in the Cruciferae.

The ovule of the Caryophyllaceae on the other hand is very different. The young ovule certainly bends, but this bend occurs near the top. It is the nucellus that is affected and not the funicle, as can be observed in *Cerastium arvense* L. (Fig. 2a, 2b). At maturity the ovule in the Caryophyllaceae is much more closely related to the orthotropous ovule of a *Polygonum* than to that of the Leguminosae or the Cruciferae: bent on one side, it is seated on its chalaza at the top of a rectilinear funicle. At the most there is a very slight bend of the vascular strand in the funicle during the development of the seed, for example, this occurs in the seed of *Heliosperma quadridentatum* (Murray) Schinz & Thellg. (Fig. 3). It would appear then that this type of campylotropous ovule which is frequently met with in the Centrospermae (Rocen, 1927) could be considered as having been derived from an orthotropous ovule: It is, in fact, an orthotropous ovule with a bent nucellus or ortho-campylotropous ovule. An abnormal ovule observed in *Heliosperma quadridentatum* confirms this point of view (Fig. 4): the outer integument is loose and forms a collar around the nucellus and inner integument, which are practically straight; the ovule is, in fact, orthotropous.

Baehni & Bonner (1953) describe another example of this type of ortho-campylotropous ovule in *Aesculus parviflora* Walt. The authors noted that the ovule was indeed campylotropous but that its base and that of the funicle are on the same axis, there is no bend. In this respect the ovule is undoubtedly orthotropous. In conclusion, it can be said that the ovule of *Aesculus parviflora* illustrated in their paper (Fig. 2) is an ortho-campylotropous ovule.

Goebel (1933) considered the ovule of *Atriplex hortensis* L. as amphitropous. Nevertheless it also is ortho-campylotropous. It displays a developmental sequence analogous to that seen in *Pisum* and *Biscutella*. As in these two amphitropy is late in its initiation, and develops only during seed formation. This ovule by analogy can thus be called ortho-amphitropous. As the ovule is seated on its chalaza, it is necessarily from this region that the basal body develops.

Discussion

Systematists frequently make use of characteristics drawn from the ovule (stages up to fertilization) or from the seed (stages after fertilization up to the ripe seed) often without stating clearly the stage under consideration. It is vital, however, to follow the development from the early to advanced stages for all essential structures to have been differentiated. For example, the seeds of *Vicia* and *Cerastium* on the one hand and those of *Pisum* and *Atriplex* on the other form two pairs which externally appear to be phylogenetically related: both *Vicia* and *Cerastium* have kidney-shaped ovules which develop into kidney-shaped seeds.

←

FIGS. 1-5 — [*cha*, chalaza; *emb*, embryo and its appendages; *fun*, funicle; *Fun*, funicular strand; *hyp*, hypostase; *mi*, micropyle; *nu*, nucellus and embryo sac; *e teg*, outer integument; *i teg*, inner integument. *Tissues: A*, epidermal and tegumentary tissues; *B*, of nucellus and embryo; *C*, other parenchymatous tissues; *D*, hypostase, collenchymatous tissues; *E*, conductive tissues (elongated elements); *F*, lignified strands. The course of strands prior to lignification is indicated by dotted lines.] Fig. 1. *Biscutella cichoriifolia* Loisel. a-c. Ovules before anthesis; dimensions: a. 220×150μ; b. 600×440μ; c. 910×620μ. d. ovule just after anthesis, dimensions 1056×990μ. Fig. 2. *Cerastium arvense* L. a. Ovules from a young bud, dimensions: 125×95μ. b. same, just before anthesis, dimensions: 250×150μ. Fig. 3. *Heliosperma quadridentatum* (Murr.) Schinz & Thellg. Young seed, dimensions: 1·0×0·7 mm. Fig. 4. *Heliosperma quadridentatum* (Murr.) Schinz, malformation of an ovule from a flower at anthesis, dimensions: 700×350μ. Fig. 5. *Paronychia serpylliflora* (Chaix) DC. Ovule at anthesis, dimensions: 400×200μ excluding the funicle.

Pisum and *Atriplex* also have reniform ovules but their seeds are spherical.

However, when taken into consideration the basic structure of such ovules, it becomes clear that *Vicia* and *Pisum* are both anatropous in their early stages while *Cerastium* and *Atriplex* are initially orthotropous and in spite of the external appearances of their seeds *Vicia* and *Pisum* are more closely related to each other than to *Cerastium* and *Atriplex* respectively. Likewise the latter two have a greater affinity to each other than to either of the former.

The very early bending of the funicle beneath the chalaza is the basic phenomenon which is characteristic of anatropy. Considerable phylogenetic significance could be attributed to this feature. The Leguminosae provide a pertinent argument in favour of this point of view: their ovules are all anatropous during the early stages of their development but only those of the Papilionaceae become ana-campylotropous during the formation of their seeds.

Campylotropy and even more so, amphitropy are phenomena which only appear in the later stages of ontogenesis and furthermore characterize more limited, subordinate groups in the same way as arils, caruncles and diverse other appendages or features of the seed may be confined to individual genera or characterize a species. Their importance in systematics is therefore generally less.

Hence it is proposed to submit a classification of ovules which takes into consideration their more intimate structure and their ontogeny. Two main groups are postulated: an orthotropous series and an anatropous one (including the intermediate hemitropous state). To each group can be referred a campylotropous modification. These are the ortho-campylotropous and ana-campylotropous ovules respectively. The formation of a basal body and consequent amphitropy is likewise possible in either group, the ovules becoming ortho-amphitropous and ana-amphitropous respectively (Fig. 6).

There is no doubt as to the value of a correct interpretation of the morphology of the ovule in systematics without, however, attributing to the characters of the ovule a dominant value. Nevertheless, these characters must be considered as stable within the families.

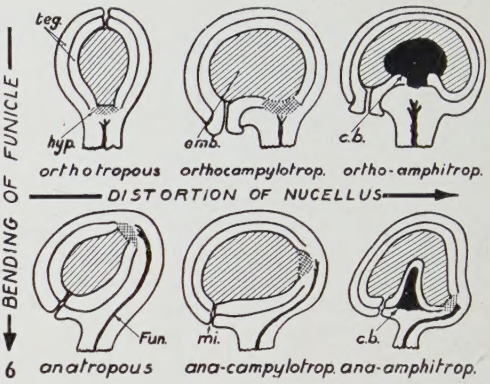

Fig. 6. (as for preceding Figs; *c.b.*, basal body). The relation between the different types of ovules. The orthotropous series above with its ortho-campylotropous and ortho-amphitropous derivatives and the anatropous series below with the corresponding ana-campylotropous and ana-amphitropous derivatives.

The advantages of the proposed classification can be illustrated by the following four examples:

1. In the case of *Aesculus*, if Baehni & Bonner's conclusions are correct, the ovule is orthotropous. This means that for *Aesculus* one cannot speak of epitropy (where the funicle is bent upwards, towards the top of the ovary) or of apotropy (where the funicular bend is downwards, towards the base of the ovary) since there is no bend in the funicle. The curvatures are entirely in the upper nucellar region. This may have considerable systematic repercussions since the distinction between the Sapindales and the Geraniales as established by Engler (1897) is based on this criterion, the former having an epitropous ovule whereas it is apotropous in the latter.

2. Conversely, if in a family (e.g. Leguminosae or Cruciferae), where the ovule is currently regarded as being campylotropous, the ovule is in fact ana-campylotropous, it might become possible to make use of the distinction between epitropy and apotropy according to the direction of the funicular bend.

3. In the case of the Centrospermae, for example, the presence of ortho-campylotropous and ortho-amphitropous ovules in the Caryophyllaceae, Basellaceae and Chenopodiaceae would provide further evidence in support of their phylogenetic relationship.

4. An exact understanding of the structure of the ovule facilitates the interpretation of certain anatomical peculiarities. For example, the single ovule of *Paronychia serpyllifolia* (Chaix) DC. is suspended at the end of a long bent funicle (Fig. 5). At first sight this ovule would appear very different from those usually observed in the Caryophyllaceae. However a careful comparision of this ovule with the ortho-campylotropous ovules of other members of the family reveals that the real funicle is very short and ends above the bend *a-b*. The long supporting stalk is placental and probably represents a relic of the central column. The thickening of the lower end of the funicle is also characteristic of a regressive vascularization and adds more weight to this interpretation.

It is thus obvious that a more detailed study of the structure and vascularization of the campylotropous ovule may furnish many important indications to the systematist. It is, therefore, intended to examine a certain number of families in detail and to publish the results as a series of papers.

Summary

It is customary to classify ovules as ortho-, ana-, campylo-, hemi-, or amphitropous. This nomenclature reflects but poorly any systematic differences or phylogenetic relationships.

A better classification can be obtained by taking into consideration the mode of development of the ovule and the organization of its vascular supply. From observation it becomes evident that the basic groups are orthotropous and anatropous. The curvatures which give rise to campylotropy are belated modifications of the basic type. Such modifications have, therefore, less phylogenetic value. It is thus possible to distinguish two basic series: the orthotropous series and the anatropous series. Both can evolve in a similar manner giving rise to similar forms: ortho-campylotropous (e.g. Caryophyllaceae) in the former series and ana-campylotropous (e.g. Leguminosae) for the latter. More extensive curvatures lead to the formation of ortho-amphitropous ovules (e.g. *Atriplex hortensis*) in the first series and to ana-amphitropous ovules (e.g. *Pisum sativum*) in the second.

I wish to tender my warmest thanks to Dr C. E. B. Bonner for his constant encouragement, his advice and criticism during the preparation of this paper and most particularly for the time and trouble involved in translating my text into English.

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DEVELOPMENT OF THE SHOOT OF *ORYZA SATIVA* L.— I. THE SHOOT APEX

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Introduction

The anatomical development of shoots of grasses is not nearly so well understood as that of many dicotyledons. There has been an overwhelming emphasis on fully developed tissues and organs, but very little on the sequence of events which culminate in their formation.

The rice plant was chosen for this investigation for two reasons: to advance our knowledge of shoot development in grasses and to increase our understanding of the ontogeny of the healthy shoot in connection with studies of growth-regulator treated plants (Kaufman, 1955). In cognizance of well-documented information on organography, gross morphology, and histology of the rice plant (Hector, 1936; Juliano & Aldama, 1937), primary consideration in this work is given to studies on ontogeny of the shoot apex, leaf, stem, and adventitious root (cf. papers II and III to follow in this series).

Previous Work

Information on the form, size, and cytohistology of angiosperm shoot apices is relatively extensive (Schüepp, 1926; Foster, 1939, 1941, 1949; Philipson, 1949; Popham, 1951; Esau, 1953; Gifford, 1954). This review primarily is concerned with studies of monocotyledon shoot apices and especially those of the Gramineae.

The form and size of the shoot apex in angiosperms vary appreciably (Herrig, 1915; Rösler, 1928; Kliem, 1937; Ball, 1941; Boke, 1941; Gifford, 1950), and within a given plant, the apical meristem has been shown to undergo changes in form during development (Noguchi, 1929; Bonnett, 1935, 1936-37, 1940, 1953; Weber, 1938; Evans & Grover, 1940; Sharman, 1942a, 1942b, 1945, 1947; Reeve, 1948; Abbe & Phinney, 1951; Barnard, 1955, 1957) as well as during a given plastochron (Schüepp, 1914; Randolph, Abbe & Einest, 1944; Gifford, 1950; Abbe, Phinney & Baer, 1951; Andersen, 1952). Rösler (1928) has indicated that within a plastochron, form changes do not occur in the vegetative apex of *Triticum vulgare*; the converse has been found for *Zea mays* (Abbe, Phinney & Baer, 1951) as well as for *Oryza sativa* (present work). Further studies on form changes in shoot apices of grasses are thus necessary before a generalization can be made.

Sharman (1942a) established a typological classification of grass apices based on relative height of the apical meristem and frequency of occurrence of primordial leaf protuberances. Under this classification are the long type, having twelve to twenty leaf primordia; the intermediate type, having five to ten primordia; and the short type, having one or two primordia.

We have few accurate quantitative data on the size of shoot apices at different stages of development. Kemp (1943) has presented a comprehensive compilation of data on heights, diameters, and ratios of height to diameter of shoot apices in a wide range of gymnosperms. Foster (1949) cites diameter measurements for various plants in different groups of angiosperms and gymnosperms. The range of dimensions is quite wide: 3500μ for *Cycas revoluta*, 700 to 900μ for *Trichocereus*, 500μ for certain palms, 130 to 200μ for various dicotyledons, and 90μ for certain grasses. Abbe & Phinney (1951)

and Abbe, Phinney & Baer (1951) have made quantitative studies on vegetative apices of *Zea mays* and graphically correlated size variations with changes in cell number and area. Stant (1954), who analysed quantitatively the growth organization of shoot apices of certain monocotyledons, suggests that the ratios

$$\frac{\text{length of rib meristem}}{\text{total length of apex}} \quad \text{and} \quad \frac{\text{width of flank meristem}}{\text{total width of apex}}$$

are the best criteria to employ in describing the shape of shoot apices.

The concept of apical initials has been widely used by investigators who have studied shoot apices of grasses (Korschelt, 1884; Douliot, 1890, 1891; Buder, 1928; Rösler, 1928; Porterfield, 1930; Kliem, 1937; Hsü, 1944; Sharman, 1945; Hamilton, 1948; Stant, 1952). In 1884 Korschelt, who made studies of ten monocotyledons, including five grasses, attempted to refute the Hansteinian concept and suggested that the apical cell concept was more applicable. He claimed that a more or less conspicuous tetrahedral cell was present in the median peripheral position of the shoot apex. Critical researches by other workers have since negated this assumption.

A number of investigators, adhering to the Hansteinian histogen concept or modifications of it (Douliot, 1890, 1891; Porterfield, 1930; Sharman, 1945; Hamilton, 1948), have repeatedly referred to apical initials. Sharman (1945) indicates that cells of the outermost tunica layer[1] in grasses are perpetuated by a poorly-defined apical initial or a group of apical initials; that subjacent to this layer may be other layers[1] and the corpus[1], each of which is perpetuated by an apical initial or a group of apical initials; and that an apical initial may occasionally divide periclinally to contribute new apical initials to two separate tiers or a new apical initial to one tier and a lineal descendant below (not in position of an apical initial, as might occur in the corpus).

Workers using the tunica-corpus concept, or modifications of it, also have described apical initials in the shoot apices of grasses (Douliot, 1890; Buder, 1928; Rösler, 1928; Kliem, 1937; Hsü, 1944; Hamilton, 1948; Stant, 1952, 1954). Some say that the apical initials occur in groups in the apical regions of tunica and corpus; others indicate that apical initials may occur as single cells. Stant (1952) further points out that divisions in groups of apical initials of the grasses are relatively infrequent in contrast with actively dividing cells in tissue of the flank and rib meristem regions. This is substantiated by relatively recent studies of Buvat (1952, 1953).

Some time after introduction of the tunica-corpus concept, cytohistologic patterns or zones were recognized in gymnosperm shoot apices (Foster, 1938; Camefort, 1951; Johnson, 1951). Later, some recognized similar cytohistologic zonation in angiosperm shoot apices (cf. Philipson, 1949; Esau, 1953; Gifford, 1954) and superimposed such interpretations upon those made in connection with the tunica-corpus concept. It seems that Popham (1951), in suggesting that we discard the tunica-corpus concept, did not recognize its continued usefulness as it evolved in relation to cytohistological interpretations of shoot apices.

Buvat (1952, 1953) and his colleagues (cf. Lance, 1957) have recently promulgated the " méristème d'attente " concept to explain cytohistologic structure and growth of shoot apices of angiosperms and gymnosperms. They visualize the tunica as being composed of a peripheral region, the " anneau initial ", where mitotic activity is intense and leaf primordia have their genesis; and an apical region, the " proméristème sporogène ", characterized by the presence of inactive cells, having a low incidence of mitotic activity. They picture the corpus as being constituted by a " proméristème réceptaculaire " to which is ascribed little cytohistologic function during vegetative growth of the apex; and a " méristème medullaire " region, composed of a rib meristem that gives rise to the pith (cf. Gifford, 1954).

1. Dermatogen, hypodermis, subhypodermis for tunica and inner core cells for corpus according to Sharman's terminology.

The " proméristème sporogène " and the " proméristème réceptaculaire " constitute the "méristème d'attente". The implication here is that the apical initial region in the vegetative shoot apex is essentially non-functional and in a " resting " state. However, Gifford (1954) thinks that the researches of Dermen, Satina, Blakeslee, and others on periclinal cytochimeras in shoot apices negate this concept of " méristème d'attente ".

Quantitative studies on the number of tunica layers in shoot apices of various monocotyledons have been summarized by Popham (1951) and later by Gifford (1954). Gifford's tabular summary indicates that the number of tunica layers varies considerably within the monocotyledons, from one family to another, within different genera of the same family, and even within the confines of a single species. Brown *et al.* (1957) report an exception in the Gramineae; namely, the occurrence of one tunica layer in most members of the Panicoideae observed and a trend toward two layers in the Festucoideae.

Variations in the arrangement of cells in angiosperm shoot apices have received much attention (Cross, 1936; Rüdiger, 1939; Boke, 1941, 1947; Reeve, 1942, 1948; Engard, 1944; Hsü, 1944; Sharman, 1945; Miller & Wetmore, 1946; Philipson, 1947; Hamilton, 1948; Popham, 1951; Thielke, 1951, 1954). Some of these workers suggest that as the vegetative shoot apex develops, layers in the tunica may become more distinct and that the number may actually increase. Many investigators indicate that the corpus may exhibit stratification at the periphery. It has also been pointed out that the number of tiers of cells in the tunica and corpus may fluctuate during individual plastochrons. Reeve (1948), for example, has suggested that the number of tunica layers is smaller after initiation than immediately preceding initiation of leaf primordia.

Stratification or layering of cells in the tunica is a consequence of absence of periclinal divisions. In apices of several monocotyledons it has been noted that the continuity of the outermost tunica layer may be interrupted by occasional periclinal divisions. These occur at the extreme tip of the apical meristem and hence cannot be ascribed to divisions associated with leaf primordia. Some of the monocots in which this phenomenon occurs include *Zea mays* (Sharman, 1940), *Agropyron repens* (Sharman, 1943), *Chlorogalum pomeridianum* (Sterling, 1944), *Ruppia maritima* and *Cymodosea nodosa* (Pottier, 1934), *Triticum vulgare* (Rösler, 1928), *Avena sativa* (Kliem, 1937), and *Tradescantia fluminensis* var. *albostricta* (Thielke, 1954). An interesting example of a shoot in which periclinal divisions occur with apparent frequency in the surface layer is *Saccharum officinale* (Thielke, 1951), suggesting the presence of a non-stable tunica.

Materials and Methods

Caryopses[2] of *Oryza sativa* L. var. " Caloro " were placed on filter paper in Petri dishes, submerged in water, and germinated under incandescent lights. Seven-day old seedlings of uniform size were selected and transplanted to one-gallon containers in the greenhouse. They were grown in water-saturated Yolo clay loam soil, ten seedlings per culture, then flooded with water to a depth of about 0·5 inches. After they were transplanted, the plants were kept in the greenhouse where they attained maturity.

Shoots and shoot segments obtained from the Petri dish and greenhouse cultures were employed both for dissection studies of living plants and anatomical investigations. The living shoots were obtained at different time intervals and sectioned serially with a sharp razor blade. The sections were placed on a slide, saturated with water, treated with various dyes (neutral red, cotton blue, basic fuchsin, Sudan IV, ruthenium red) and I_2KI, and observed under a dissecting binocular microscope.

Quantitative and qualitative data on leaf and shoot apex development were obtained for other studies with living

2. Donated to the writer by the Agronomy Division of the University of California. The seed was derived from genetically pure stock of variety, " Caloro ", grown at the Rice Experiment Station, Biggs, California.

shoots. Measurements of lengths of laminae and sheaths from shoots of different ages were made in one phase of this investigation. In another phase the shoots were dissected for measurement and illustration of shoot apices.

For anatomical studies shoot segments were killed in a solution of Craf III and "Nonic 218" surfactant (0·1 per cent). The latter was included in order to accelerate the aspiration process, which required 30 to 60 minutes, depending upon the amount of air space in the tissues. The tertiary butyl alcohol method described by Johansen (1940) was employed in the dehydration process. The shoot segments were embedded in Fisher's "Tissuemat" (melting point range of 52-54°C). The material was cut at 6-10 μ. Segments in blocks were softened in a solution containing 20 ml glycerine, 1 ml "Nonic 218" surfactant, and 70 ml water for 5 ± 2 days prior to sectioning. This softening process, the use of microtome knives, and cutting at low temperatures reduced tearing and scratching of ribbons.

The tannic acid-iron chloride staining schedule of Foster (1934) was employed. Several modifications of this schedule included lengthening the time of immersion in tannic acid to 12 minutes, using a methyl cellosolve-alcohol solution of safranin (Johansen, 1940), and staining with fast green for a period of three minutes prior to immersion of slides in xylene at the end of the schedule.

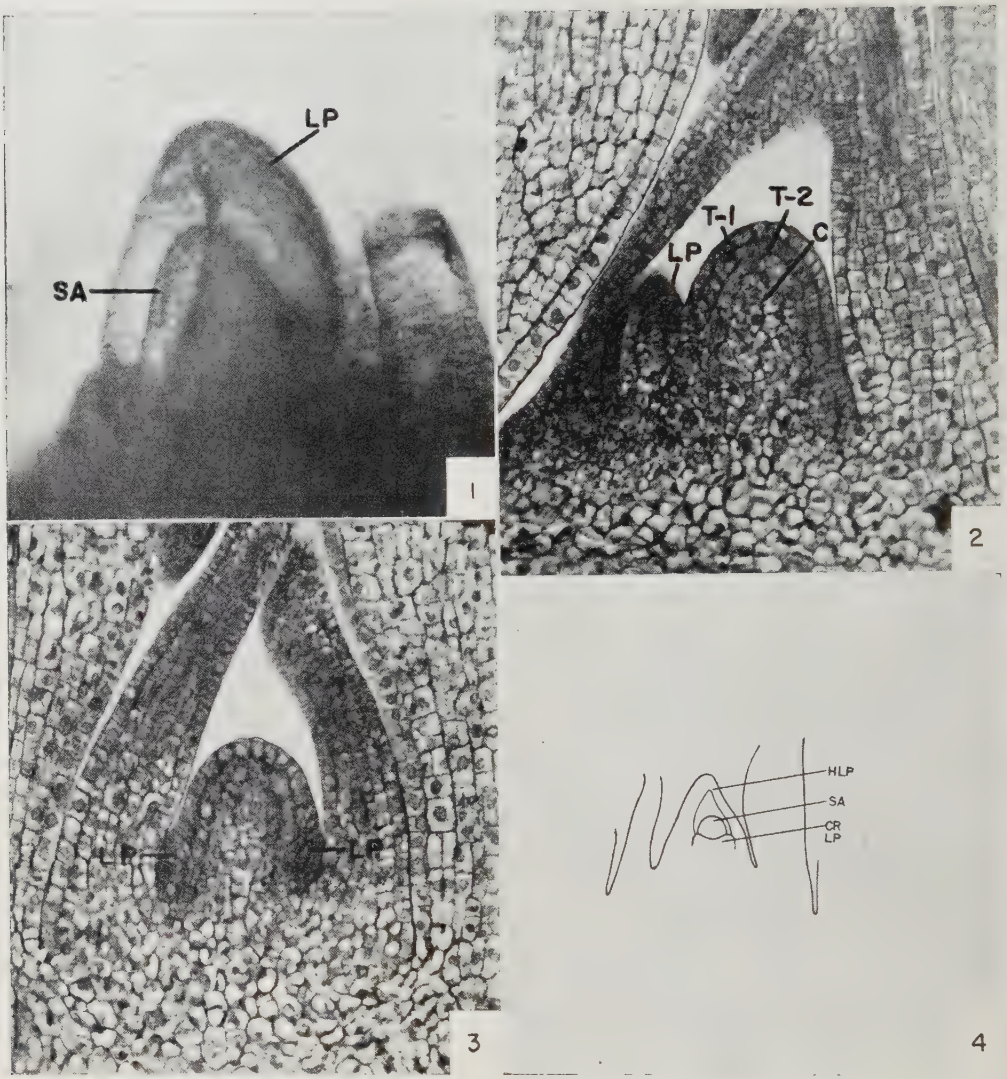
Changes in Form and Size of the Shoot Apex during Ontogeny

The vegetative shoot apex of the rice plant is a conical dome, obtusely rounded at the summit and steeply sloped along the sides (Figs. 1, 4). This apical meristem is one of the short types as described by Sharman (1945).

The apical meristem of the embryo and seedling is a narrow cone, having a slender rounded tip (Fig. 5). The vegetative apex becomes larger during successive plastochrons, primarily because of an increase in volume growth of the corpus and surface growth of the tunica. Furthermore, the number of layers in the tunica increases from one to two, and the corpus develops a tendency towards stratification (Figs. 5-7). During an individual plastochron, changes in form also occur. The shoot apex is shorter and narrower during the minimal area phase of a plastochron (just after leaf initiation, Fig. 9) and longer and broader during the maximal area phase (just before and during first stages of leaf initiation, Fig. 11). During transition from vegetative to reproductive stages, enlargement of the apical meristem is particularly marked. At this time it becomes a massive structure with a broad curved tip and long steeply sloped sides (Fig. 13). However, as soon as primary branch primordia become evident, the main apical dome, now in the early reproductive stage, assumes a broad short form with an obtusely rounded tip (Fig. 14). The form of apices of primary and secondary branches (rachillae) and spikelets is approximately the same as that of the main apical inflorescence meristem (Figs. 15-21). The spikelet apex, however, tends to be shorter and broader than that of a rachilla (cf. Figs. 18, 21).

In order to obtain quantitative data on the size of the shoot apex of rice, many sagittal and transverse sections of apices were studied at different stages of development. To obtain dimensions of length and width, only that part of the apical meristem was measured which showed no evidence of primordial leaf and floral appendage protuberances; and the width was measured just above the level of initiation of a leaf. Table 1 summarizes the average lengths and diameters of apices at different stages of development of the rice plant.

Several facts are explicit in Table 1: (i) the shoot apex of the seedling is long and narrow, the length exceeding the width; (ii) the lengths and widths of vegetative apices, with some exceptions, increase during successive plastochrons; (iii) the widths of these apices are considerably greater than their lengths; (iv) during transition to reproductive phase the length of the apex increases greatly in proportion to the transverse diameter so that the length actually surpasses the width in the early panicle apex (prior to initiation of primary branch



FIGS. 1-4 — (*C*, corpus; *CRLP*, crescent-shaped leaf primordium; *HLP*, hood-shaped leaf primordium; *LP*, leaf primordium; *SA*, shoot apex; *T*-1, *T*-2, tunica layers 1 and 2 respectively). Fig. 1. Portion of a living shoot. × 625. Fig. 2. Median l.s. of vegetative shoot apex cut perpendicular to the planes of the leaf blades. × 560. Fig. 3. Median l.s. of vegetative shoot apex cut parallel to the planes of the leaf blades. The densely-stained regions are parts of a recently initiated leaf primordium. × 560. Fig. 4. Drawing from portion of living shoot as viewed in plane parallel with planes of leaf blades. × 325.

primordia); (v) when rachillae primordia begin to elongate rapidly, the lengths of the apices of these branches also exceed the transverse diameters; (vi) the diameters of early panicle (after initiation of primary branch primordia) and spikelet apices are greater than the lengths.

Since the dimensions of vegetative shoot apices of rice plants of the same age were found to vary considerably, the writer was interested in determining whether this variation might be caused by changes in size of the apex during a single plastochron. The lengths and diameters of

TABLE 1 — LENGTHS AND WIDTHS OF VEGETATIVE AND REPRODUCTIVE SHOOT APICES OF THE RICE PLANT

Stage of plant development	Age of plant in days	Number of apices measured	Average length in microns	Average diameter in microns
Seedling	7	3	57·6	44·9
Early vegetative	15	4	41·4	48·4
Middle vegetative	36	6	38·3	54·0
Late vegetative	50	7	47·8	67·9
Late vegetative	64	6	57·3	68·3
Transition	64	1	64·0	72·0
Early panicle*	64	1	144·0	83·2
Early panicle**	64	1	36·8	70·9
Rachillae primordia	71	2	93·6	70·4
Spikelet primordia	78	2	35·2	70·4

*Prior to the initiation of primary branch primordia.
**Just after the initiation of primary branch primordia.

TABLE 2 — LENGTHS AND WIDTHS OF VEGETATIVE SHOOT APICES DURING A SINGLE PLASTOCHRON

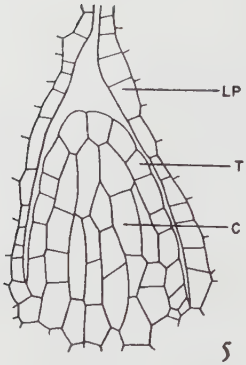
Stage of plastochron	Stage of development of leaf primordium	Age of plant in days	Length of apex in microns	Transverse diameter of apex in microns
Transition to maximal area phase	Cowl over apex	36	38·4	45·1
Maximal area phase	Primordial leaf protuberance	36	45·9	55·6
Minimal area phase	Crescent-shaped protuberance	36	25·1	45·1
Minimal area phase	Cowl around base of apex	36	25·1	41·8

apices in different phases of the same plastochron are summarized in Table 2.

Table 2 shows that the dimensions of the vegetative apex may change appreciably during a single plastochron. The same holds true for *Zea mays* (Abbe, Phinney & Baer, 1951). The trend in the rice plant appears to be a decrease in length and width (more so in length than in width) as the apex proceeds from the maximal area phase to the minimal area phase of a plastochron.

Cytohistology of Vegetative and Reproductive Shoot Apices

The most conspicuous cytohistological features of vegetative and reproductive apices of the rice plant are as follows. In the embryo and seedling the cells of the uniseriate tunica and corpus are stained more or less homogeneously. Vacuolation is not conspicuous in these regions; it is more apparent in the developing pith (rib meristem zone) subjacent to the corpus. In addition cell walls are thin, nuclei are large in relation to cell volume, and cytoplasm is relatively uniform in density in both tunica and corpus. As seen in longitudinal section, the corpus cells tend to be somewhat elongated, whereas those of the tunica are isodiametric (Fig. 22). During subsequent plastochrons both tunica and corpus cells in vegetative shoot apices appear more or less isodiametric (Figs. 2, 3, 6-13). The



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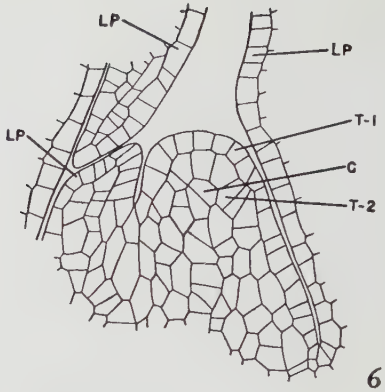

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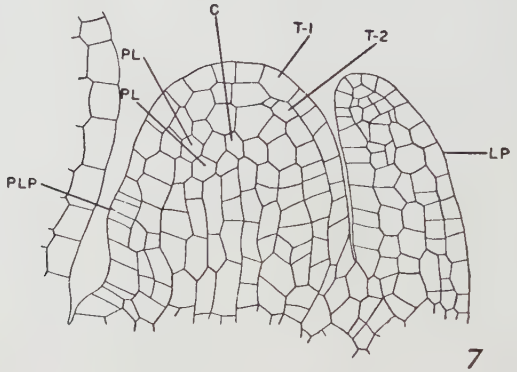

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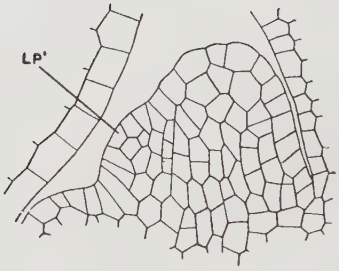

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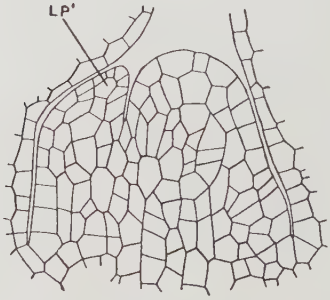

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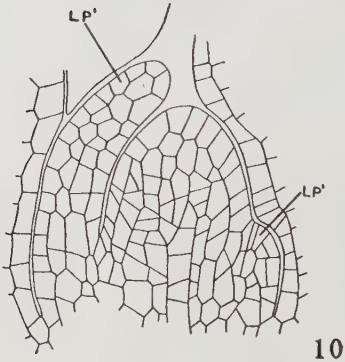

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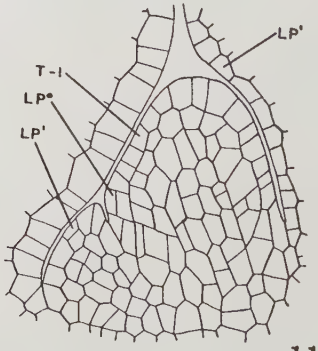

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FIGS 5-11.

degree of vacuolation also increases in cells of both zones, especially near the base of the corpus and in the uppermost region of the tunica (cf. Figs. 22, 23 and observe still older vegetative apices in Figs. 2, 3). In later stages of development of the vegetative shoot apex, starch is deposited in nodal regions below the corpus and eventually in the base of the corpus. In young panicle apices cells of the corpus increase in size and vacuolation, whereas cells of the tunica remain about the same size and become more densely cytoplasmic than in the late vegetative stage (Fig. 24). The change in degree of vacuolation of corpus cells is first evident in the central region and gradually spreads centrifugally to the peripheral regions. These observations also apply to rachillae apices. In spikelet apices, however, both tunica and corpus cells appear densely cytoplasmic.

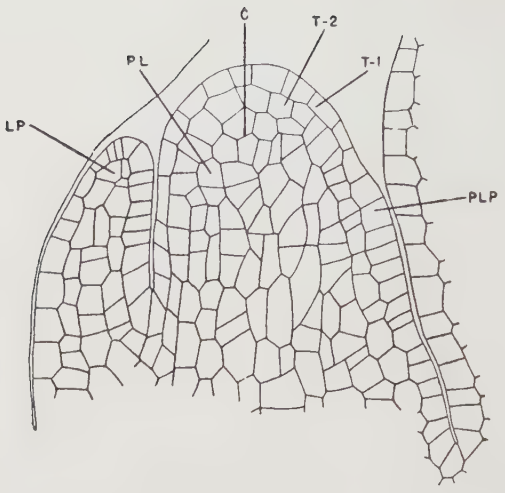
A study of stratification and the occurrence of apical initials in vegetative and reproductive apices was made concurrently with the cytologic investigation of these apices. Vegetative apices of embryos and seedlings have a uniseriate tunica and a narrow corpus (*see* seedling apex in Fig. 5). Peripheral layering in the corpus was not evident in these apices. In apices of young vegetative plants the tunica is biseriate. Further studies of this difference in the number of strata in the tunica showed that the biseriate condition probably results from the addition to the tunica of a layer that has its genesis in the corpus. The biseriate tunica persists through successive plastochrons in the development of the vegetative apex (Figs. 6, 7, 12). During later stages the corpus tends to develop one or two peripheral layers in which anticlinal divisions predominate (Figs. 7, 12). There is no evidence that the number of tunica layers increases during leaf initiation and decreases after leaf initiation (Figs. 8-11). At the transition phase of development of the apical meristem the two tunica layers persist, although T-2 is not as distinct; and peripheral layering in the corpus is still evident (Fig. 13).

The apices of primary and secondary branch (rachillae) primordia have an unstable biseriate tunica derived from the biseriate tunica of the transition apex and a corpus usually devoid of peripheral layering (Fig. 18). In the biseriate tunica of these apices periclinal divisions may occur in T-1 and/or T-2. This activity, together with divisions in the corpus, is responsible for the initiation of spikelet primordia.

In spikelet apices the tunica is uniseriate, and the corpus zone is shallow in comparison to the size of the corpus of vegetative apices (Figs. 20, 21). As the spikelet develops, its floral apex is finally transformed into glume, lemma, palea, anther, and pistil primordia. Although Juliano & Aldama (1937) have treated organogenesis of the rice spikelet in considerable detail, further study is necessary to elucidate the cytohistologic development of the spikelet apex and its appendages. Also, we need to determine more fully the stability and manner of origin of the tunica and corpus in rachillae and spikelet apices.

←

FIGS. 5-11 — (*C*, corpus; *LP*, leaf primordium; *LP°*, new leaf primordium; *LP*^{1}, youngest leaf primordium; *PL*, peripheral layer; *PLP*, primordial leaf protuberance; *T*, uniseriate tunica; *T-1*, T-*2*, tunica layers 1 and 2 respectively). Median l.s. of vegetative shoot apices cut perpendicular to the planes of the leaf blades; apices were derived from shoots of different ages. Fig. 5. Shoot apex of seven day old seedling. × 595. Fig. 6. Shoot apex of thirty-six day old plant. × 595. Fig. 7. Shoot apex of a fifty day old plant. × 595. Figs. 8-11. Median l.s. of vegetative shoot apices cut perpendicular to the planes of the leaf blades. These apices were derived from thirty-six day old plants and represent different stages of development during one plastochron. They are depicted here to show variations in form and size preceding and following leaf initiation. × 470. Fig. 8. Minimal area phase of the plastochron; *LP*^{1} forms a crescentic protuberance at one side of apical meristem. Fig. 9. Minimal area phase of plastochron; *LP*^{1} encircles base of apical meristem but does not overtop it. Fig. 10. Maximal area phase of plastochron; *LP*^{1} forms cowl over shoot apex. Fig. 11. Maximal area phase of plastochron; *LP*^{1} overtops shoot apex and is no longer in cowl stage. Locus of *LP°* is defined by products of periclinal divisions in *T-1*.



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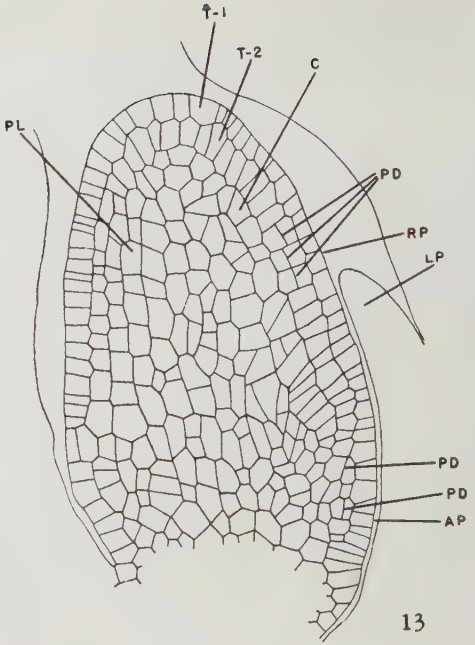

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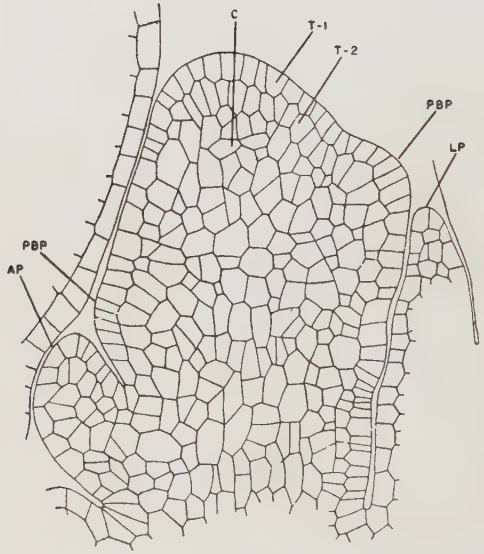

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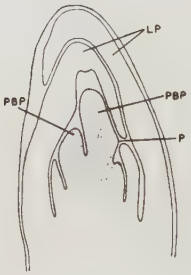

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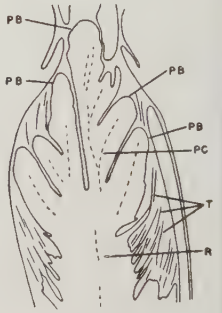

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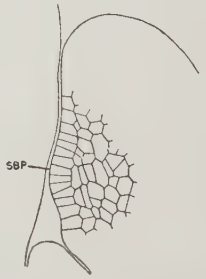

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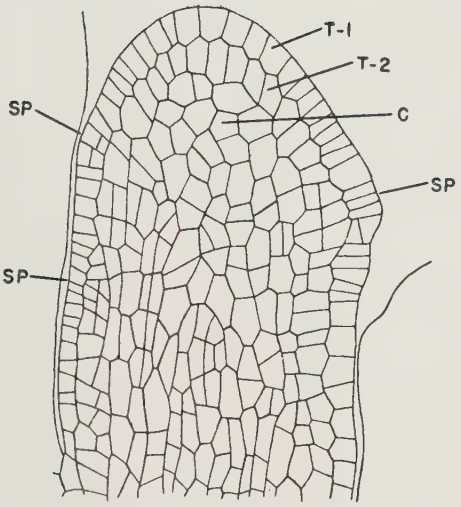
FIGS. 12-17 — (*AP*, appendage primordium; *C*, corpus; *LP*, leaf primordium; *P*, panicle inflorescence; *PB*, primary branch; *PBP*, primary branch primordium; *PC*, procambial strands; *PD*, periclinal divisions; *PL*, peripheral layer; *PLP*, primordial leaf protuberance; *R*, rachis; *RP*, rachilla primordia; *SBP*, secondary branch primordium; *T*, trichomes; *T-1*, *T-2*, tunica layers 1 and 2 respectively). Figs. 12-14, 17. Median l.s. from shoots cut perpendicular to the planes of the leaf blades; Figs. 15, 16. Median l.s. from shoots cut parallel with the planes of the leaf blades. All figs. represent portions of shoots from sixty-four day old plants. Fig. 12. Vegetative shoot apex at about transition from vegetative to reproductive stages of development. × 667. Fig. 13. Shoot apex at transition stage of development (vegetative to reproductive stages); the apex is still enclosed by leaf primordium. × 708. Fig. 14. Young inflorescence apex just after transition stage of development. × 760. Fig. 15. Panicle inflorescence and surrounding leaf primordia. × 92. Fig. 16. Inflorescence with many primary branches (rachillae). × 90. Fig. 17. Portion of primary branch tip, where a secondary branch primordium is being initiated; this initiation is similar to that of axillary buds of the vegetative plant. × 534.



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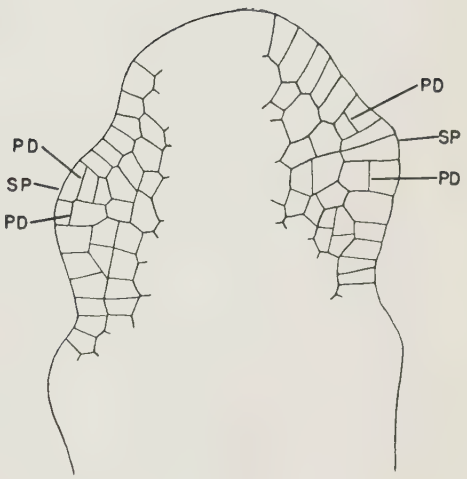

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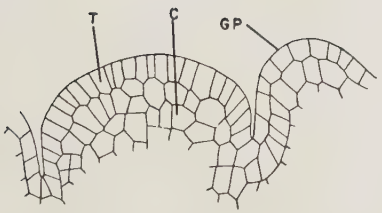

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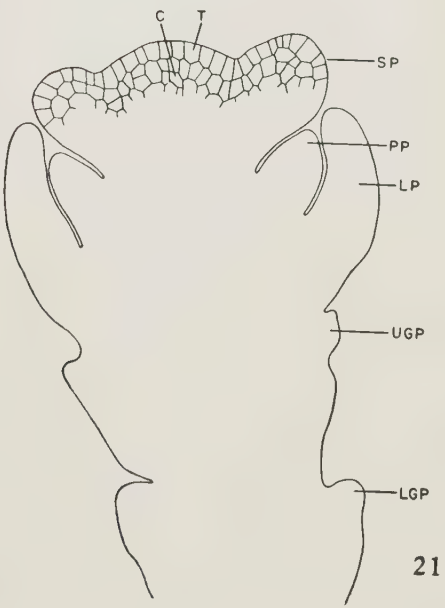

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FIGS. 18-21 — (*C*, corpus; *GP*, glume primordium; *LGP*, lower glume primordium; *LP*, lemma primordium; *PD*, periclinal division; *PP*, palea primordium; *SP*, spikelet primordium in Figs. 18, 19, stamen primordium in Fig. 21; *T*, uniseriate tunica; *T-1*, *T-2*, tunica layers 1 and 2 respectively; *UGP*, upper glume primordium). Figs. 18, 19. Median l.s. of inflorescence apices cut perpendicular to the planes of the leaf blades (Fig. 18) and parallel to these planes (Fig. 19). × 630. Fig. 18. Young secondary rachilla apex and three spikelet primordia associated with axis below it. Fig. 19. Secondary rachilla primordium, illustrating later stages in development of spikelet primordia. Figs. 20, 21. Median l.s. of spikelets cut parallel with the planes of the leaf blades. Fig. 20. Young spikelet apex with uniseriate tunica and shallow corpus; anticlinal divisions predominate in peripheral layer of corpus. × 520. Fig. 21. Later stage in spikelet development, depicting spikelet apex and various appendages of the spikelet. × 370.

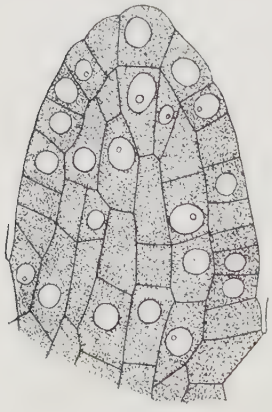
Regarding apical initials, the writer could not determine exactly which cells were apical initials in the apices he examined by virtue of the fact that these cells do not appear morphologically distinct when compared with their immediate derivatives (Figs. 2, 3).

Discussion

The quantitative morphogenetic study of Abbe & Phinney (1951) and Abbe, Phinney & Baer (1951) on vegetative shoot apices of *Zea* have demonstrated definite and statistically significant changes in the form of the apex during and within successive plastochrons, which could be correlated with cell number, area, and size of the shoot apex and with leaf initiation. The present study on the rice shoot apex, though less detailed than that on *Zea*, reveals that ontogenetic changes may be traced through the vegetative and the reproductive stages. The rice shoot apex shows rather pronounced changes in the architecture of vegetative and floral apices during successive plastochrons and subtle form changes in the vegetative apex during a given plastochron. During a single plastochron, in plants 36 days old, a decrease in length and width of the shoot apex occurs during progression from the maximal area phase to the minimal area phase of a plastochron.

Sharman (1942a) classified shoot apices of grasses by using relative height of the apical dome and frequency of occurrence of leaf promordia. He classifies the rice shoot apex as an example of the short type. My studies corroborate this finding with one reservation. In comparison with other grasses, the shoot apex of rice cannot be considered short for *all* stages in its development. Young vegetative apices are relatively short, but older vegetative and transitional apices are relatively long.

When size of shoot apices is correlated with developmental stages of the plant, a considerable range of variability in shoot apex size is observed in the same species. For example, data in Table 1 reveal that the size of the shoot apex in rice varies from 35 to 144 μ in length and 45 to 83 μ in width. Such variation is directly correlated with different stages of development of vegetative and reproductive apices. For a complete picture of shoot apex morphology, quantitative measurements of apices of vegetative and reproductive shoots at different stages of development are indispensable. Furthermore, the part of the shoot tip considered to be the shoot apex should be clearly defined. Some investigators include the smallest primordial leaf protuberance in measuring the shoot apex; others do not (cf. Esau, 1953). In this study the



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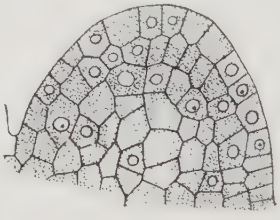

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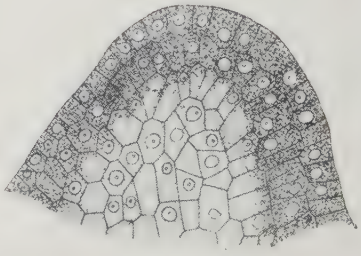

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FIGS. 22-24 — L.s. of shoot apices, depicting certain cytohistologic features. Fig. 22. Apical meristem of a three day old seedling. The cells have cytoplasm of fairly uniform density, and the corpus cells are elongated. × 800. Figs. 23, 24. Vegetative apex of a thirty-eight day old plant and inflorescence apex of a sixty-four day old plant, respectively. Vacuolation in the corpus cells and relatively high cytoplasmic density of the tunica cells are conspicuous features of these apices. × 600.

FIGS. 25-28 — (Zone 1 in Figs. 25, 28, densely cytoplasmic, approximately isodiametric, anticlinally dividing cells of uniseriate tunica; zone 1 in Figs. 26, 27, similar cells as above but pertaining to tunica layer 1; zone 2 in Figs. 25, 28, densely cytoplasmic, approximately isodiametric, anticlinally and periclinally dividing cells of the corpus; zone 2 in Figs. 26, 27, densely cytoplasmic, approximately isodiametric, anticlinally dividing cells of tunica layer 2; zone 3 in Fig. 26, densely cytoplasmic cells of peripheral layer of corpus in which anticlinal divisions predominate and periclinal divisions occur occasionally; zone 3 in Figs. 27, 28, vacuolating, approximately isodiametric, anticlinally and periclinally dividing cells of the corpus; zone 4 in Fig. 26, similar to cells in zone 3 in Figs. 27, 28 but pertaining to central part of corpus only). Diagrams depicting cytohistologic zonations in the vegetative and reproductive apices of the rice plant. Fig. 25. Vegetative shoot apex of a seedling. Fig. 26. Older vegetative shoot apex. Fig. 27. Young inflorescence apex and subjacent panicle rachis region. Fig. 28. Apex of spikelet primordium.

shoot apex was treated as the part of the shoot above the youngest discernible leaf primordium.

The number of tunica layers changes during the development of the rice plant. Vegetative apices of embryos and seedlings have a uniseriate tunica; in young vegetative (just after the seedling stage) and older vegetative apices, the tunica is biseriate. The biseriate condition persists in the transition apex (just prior to development of the inflorescence apices) and in primary and secondary rachillae apices but is uniseriate in spikelet apices. Thielke (1951), who studied the rice shoot apex only during a part of the vegetative

stage, indicated that rice probably has a biseriate tunica.

The data on planes of cell division, layering, and overall cytohistological pattern in reproductive apices of rice suggest that the main premises of the tunica-corpus concept are as applicable to these apices as to vegetative apices. The differences observed between vegetative and reproductive apices such as duration of activity, general form, size of the corpus zone, and degree of vacuolation might be interpreted as being of qualitative nature. These differences do not seem to be sufficiently fundamental to warrant consideration of vegetative and reproductive apices as irreducible types in the sense of Grégoire (1938).

Is it possible to characterize the shoot apex of the rice plant on the basis of its cytological characteristics ? I believe that this is feasible in view of the data presented on pages 233 and 235 although it appears that different schemes have to be employed to characterize the shoot apices of a seedling, an older vegetative shoot, and a reproductive shoot. The drawings presented in Figs. 25-28 interpret the four cytohistologic zonation patterns that were recognized in the rice plant.

The present study, though far from covering all aspects of apical growth, shows the importance of a multi-aspect approach to investigations on shoot apices. It gives evidence of ontogenetic variation in size and architecture of the shoot apex and, therefore, stresses the necessity of relating form and size of apices with stage of development of the plant.

Summary[3]

Vegetative and reproductive apices of *Oryza sativa* L. have a uniseriate or a biseriate tunica and a corpus varying in depth. These apices change in form and size during a single plastochron and from plastochron to plastochron.

This paper is a revised and condensed part of a thesis submitted to the Graduate Division of the University of California in partial fulfilment of the requirements for the degree of Doctor of Philosophy. The writer wishes to express his sincere appreciation to Professor Katherine Esau, University of California, Davis, California, for inspiration, guidance, and constructive criticism throughout the course of the investigation and in the preparation of this manuscript. He is also indebted to Professors Adriance S. Foster, Ernest M. Gifford, Jr., and Louis K. Mann of the University of California for helpful suggestions and comments.

3. For résumé on shoot development in *Oryza*, see summary of paper III to follow in this series.

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SHOOT PRODUCTION IN CULTIVATED TEA (*CAMELLIA SINENSIS* L.)—I. APICAL ACTIVITY AND RADIAL GROWTH

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Introduction

Tea is made from the young shoots of *Camellia sinensis* L. The Assam variety of this plant (var. *assamica* Masters) is commonly cultivated in the plains of north-east India. It is a small tree, pruned into the form of a low bush when cultivated. Shoots in a standard state of development, consisting usually of two leaves, are *plucked* at regular intervals, normally seven days. Repeated regeneration of shoots results in a surface like that of a clipped hedge. It is customary in north-east India to maintain a strictly horizontal surface at a convenient height above ground level. Field trials had shown that the efficiency of the system is conditioned by the height of the plucking surface above the bush-frame, an arbitrary height of 20 cm had been found to be generally satisfactory for the Assam variety of tea. Investigations now to be described were conducted with the object of relating practice to principles. Two lines of investigation were possible: one was to study similar shoots plucked at comparable morphological horizons, and from their behaviour advance possible reasons for the behaviour of the plucked bush; alternatively, the behaviour of the plucked bush, which is the integration of numerous non-comparable shoots, could be studied as a whole, and from this it might be possible to infer certain principles. The latter course was chosen. It will be shown that this has disclosed important morphological principles.

Structure of the Bush — A cultivated tea bush has a permanent frame the ultimate branches of which can be called sticks. When the bush is pruned, the cut ends of the sticks lie in a horizontal plane. Pruning is repeated annually in north-east India, generally in the months of December and January, when growth is at a minimum. All top-growth of the past season is removed with the exception of *stubs*, 1·5 to 3·0 cm in length, of selected well grown stems, which remain on the bush as length increments to the sticks. All the next season's shoots of importance arise from dormant buds on the stubs. It is convenient to call these new shoots *primaries*.

When the primaries reach the desired height, they are *tipped* — that is, their apices are broken off at a fixed height measured from the top of the stub. This operation may extend over a few weeks and, depending on tipping height, some apices may become permanently dormant below the tipping level. After a primary shoot has been tipped, new shoots arise from the axillary buds and when these shoots grow above the tipping level, they are plucked. Tipping is merely the initial plucking. The green weight of plucked shoot is designated *yield*.

A primary is not tipped until it has unfolded at least one leaf above the predetermined level. In practice more growth might be made above the required level and it might become necessary to break off a piece of stem carrying more than one leaf. Subsequent plucking, however, would be at set intervals with the object of producing standard shoots. The interval is commonly seven days and the shoot nominally bears two leaves. Morphological details of the system are given by Wight (1955).

The apex of a tea shoot, which is not tipped, eventually forms a resting bud. When growth is resumed, caducous cataphylls leave scars on the stem (bud trace) which mark the distal limit of a *flush*. A primary may make only one flush or a succession of flushes in the course of a growing season. Axil buds on a flush do not form *lateral shoots* until the terminal bud (dormant apex) resumes active growth. The number of laterals is variable, but they always arise from the distal leaf-axils of a flush. If a flush is broken off at any point, then laterals are formed immediately below the break by buds which have artificially become distal. The object of tipping is to cause the formation of laterals at the tipping level, but if this coincides with an horizon of dormant buds, then laterals could be expected naturally at that point.

Design of the Experiment

Ordinary tea populations are genetically very mixed, and observations on such populations need to include large numbers of bushes. As numerous simultaneous observations were required for the present investigation, it became imperative to use homogenous material. A clone was, therefore, used. At the time of observation this clone (16/10/22) was 15 years old and was considered to be sufficiently representative of much of the tea cultivated in the plains of north-east India. A plot of 24 bushes, grown in full sun, was used for the trial. The bushes in the plot were spaced five feet apart and manured with ammonium sulphate at the rate of 45 kilos nitrogen per hectare. The plot was subdivided into three sub-plots of eight bushes each. Bushes within each sub-plot were assigned the following tipping heights at random: 5, 10, 15, 20, 25, 30 and 35 cm; the eighth bush was neither tipped nor plucked.

The bushes had been pruned in December and were tipped on the 3rd March. At that date it was necessary to remove more growth from some treatments (tipping heights) than others. Subsequently the bushes were plucked every fifth day, and all growth removed, whether from the primaries or laterals, was regarded as ' yield ' or ' plucking '. A five day plucking cycle is not usual in practice but was chosen because it increases the uniformity of the plucked shoots. Shoots with more than one leaf and all dormant (*banjhi*) shoots were removed at the plucking level or at the basal cataphylls, if they were above the plucking level. This is customary, the basal cataphylls being necessary for regeneration. This procedure caused a gradual rise of the

plucking level of the order of five cm in one season. Plucking ceased on 22nd December and the bushes, inclusive of the unplucked, were pruned between the 22nd and 26th December.

Shoots were categorized into types, to which reference will be made in later papers, and weighed and counted at each plucking. The rate of growth of successive orders of laterals and the increase in girth of representative parts of the bush-frame were also studied. After recording the green weight, the prunings were stored in the laboratory for counting the number of lateral shoots of the different orders.

Experimental Results

PLUCKING WEIGHT — Although the bushes belonged to the same clone and were all of the same age, yet there was a good deal of variation in size, some of which could be attributed to border effects. This variation was recorded, before the treatments were imposed, as number of sticks in the pruned surface and as area of the pruned surface (Table 1). Subsequently the weight of shoots plucked from each bush was found to be closely correlated to number of sticks per bush ($r = 0{\cdot}88$; $P < 0{\cdot}001$). Bush yield was, therefore, adjusted on this basis by the method of co-variance. The adjusted yields, recorded in Table 2, are significantly different.

The maximum yield was obtained from bushes tipped at 15 cm and this yield was significantly higher than the yields at 5 cm and 35 cm. Raising the tipping height from 10 cm to 30 cm did not make any statistically significant difference to the yield. Figure 1, however, suggests a real depression of yield between 15 cm and 25 cm.

TABLE 2 — PLUCKING WEIGHT PER BUSH BEFORE AND AFTER ADJUSTMENT ON THE NUMBER OF STICKS

Tipping height	Plucking weight in kg.	
	Observed	Adjusted
5 cm	2·46	2·24
10 ,,	2·39	2·46
15 ,,	2·87	2·91
20 ,,	2·33	2·48
25 ,,	2·49	2·66
30 ,,	2·57	2·53
35 ,,	2·25	2·08

L.S.D. at $P = 0{\cdot}05$: 0·51 kg. for adjusted yields.

PRUNING WEIGHT — The weight of prunings was also adjusted on the initial number of sticks. Treatment differences are significant (Table 3). Apart from suggestions of two maxima at 15 cm and 30 cm, there is a gradual increase of pruning weight with height of tipping (Fig. 1). This is to be expected, but more importance attaches to the data as expressed in Table 4, in terms of the ratio of plucking weight to pruning weight and the sum of the two weights. The latter, apart from radial increments to the permanent bush-frame, approximates to the total " top-growth " of the bush.

In these terms, top-growth increases with tipping height, the greatest top-

TABLE 1 — AREA OF PRUNED SURFACE AND NUMBER OF STICKS PER BUSH BEFORE COMMENCEMENT OF THE EXPERIMENT

Tipping height	Area of pruned surface in square metre			Number of sticks		
	Bush 1	Bush 2	Bush 3	Bush 1	Bush 2	Bush 3
5 cm	0·752	1·080	0·924	89	137	150
10 ,,	0·882	0·730	0·826	128	84	96
15 ,,	1·099	0·985	0·989	141	81	90
20 ,,	1·180	0·684	0·680	133	74	81
25 ,,	1·133	0·686	0·922	113	76	94
30 ,,	1·182	1·150	0·765	104	154	74
35 ,,	1·150	1·263	1·101	116	146	102
Not tipped	0·916	0·805	0·633	99	87	90

TABLE 3 — PRUNING WEIGHT PER BUSH ADJUSTED ON THE NUMBER OF STICKS

Tipping height	Pruning weight in kg.
5 cm	1·16
10 ,,	1·74
15 ,,	2·87
20 ,,	3·00
25 ,,	3·76
30 ,,	4·72
35 ,,	4·55
Not tipped	9·39

L.S.D. at P=0·05: 1·43 kg.

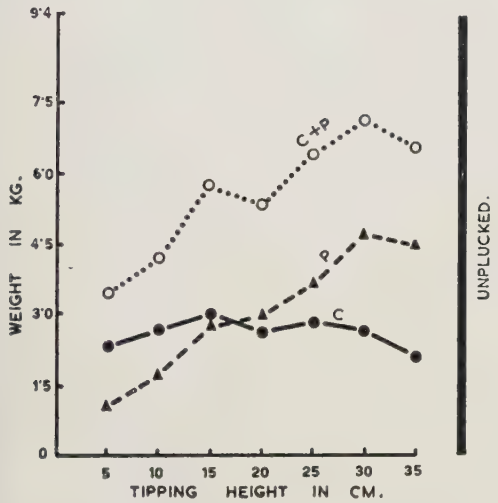

Fig. 1 — Plucking weight (C), pruning weight (P) and top-growth (C+P) per bush at different tipping heights. The vertical line of the right indicates the pruning weight of an unplucked bush.

growth being made by bushes which were neither tipped nor plucked. Top-growth is plotted against tipping height in Fig. 1, the curve suggesting two maxima, one at 15 cm and another at 30 cm. Thus top-growth and yield of plucked shoots tend to maxima at tipping heights of 15 cm and 30 cm. In later sections it will be shown that these two heights are associated with the dormancy horizons of the primary shoots.

Number of Primary Shoots — Table 5 gives the number of primary shoots per

TABLE 4 — RELATION BETWEEN PLUCKING AND PRUNING WEIGHTS AND TOP-GROWTH PER BUSH

Tipping height	Top-growth in kg.	Plucking wt. / Pruning wt.	Top-growth as per cent untipped
5 cm	3·40	1·92	36
10 ,,	4·20	1·41	45
15 ,,	5·78	1·01	62
20 ,,	5·48	0·83	58
25 ,,	6·42	0·71	68
30 ,,	7·25	0·54	77
35 ,,	6·63	0·46	71
Not tipped	9·39	—	100

TABLE 5 — NUMBER OF PRIMARIES PER BUSH ADJUSTED ON THE NUMBER OF STICKS

Tipping height	No. of plucked primaries	No. of unplucked primaries	Total of plucked and unplucked
5 cm	219	4	223
10 ,,	178	4	182
15 ,,	162	5	167
20 ,,	138	5	143
25 ,,	136	16	152
30 ,,	118	13	131
35 ,,	88	14	102

L.S.D. at P=0·05: 29 for plucked primaries

bush after adjustment for initial number of sticks. The data, therefore, can be interpreted as primaries per stick. The number of plucked primaries varies inversely as tipping height, the treatment differences being highly significant.

The growth in length of some primary shoots ceases before they reach the tipping level, more so when tipping is done at a high level. The number of such unplucked primaries is shown in the third column of Table 5. This number being very small in comparison to the number of plucked primaries, the inverse relation mentioned in the previous paragraph holds generally for the total of plucked and unplucked primaries. The data, therefore, lead to the conclusion that low tipping of primaries, that have originated from dormant buds on the sticks, forces an additional

number of dormant buds to become primaries.

Yield per Primary Shoot — The number of primary shoots is inversely related to the yield and top-growth associated with a single primary (Table 6).

These inverse relations might be supposed due entirely to partition of growth among the primaries. However, significant treatment differences in the weights of plucked shoots (Table 2) and top-growth (Table 4) and the steadily diminishing ratios of yield to top-growth per primary (Table 6) would not support this interpretation.

Data derived from Table 6 and plotted in Fig. 2 show that 15-20 cm is a critical range of tipping height. The rate of yield increment per primary differs on either side of this range. Yield measures the regeneration of axil shoots that follows decapitation of a primary; and thus it is possible to say that certain critical shoot heights determine distinct phases of potential regeneration.

Number of Leaves — It must be supposed that the number of leaves carried by the primary shoots is a factor of importance in determining yield and top-growth. The average number of leaves below the tipping level is recorded in Table 7. The data are plotted in Fig. 3 which shows that the number of leaves is strictly proportional to the tipping height. Therefore, the relations already demonstrated between yield and top-growth, and tipping height, hold also in respect of number of leaves; and thus the basal scale of Fig. 1 can be regarded also as a scale of leaf frequency.

TABLE 6 — PLUCKING WEIGHT AND TOP-GROWTH PER PRIMARY

Tipping height	Plucking weight in gm.	Top-growth in gm.	Plucking wt. / Top-growth
5 cm	10·2	15·5	0·66
10 ,,	13·8	23·6	0·58
15 ,,	18·0	35·7	0·50
20 ,,	18·0	39·7	0·45
25 ,,	19·6	47·2	0·42
30 ,,	21·4	61·4	0·35
35 ,,	23·6	75·3	0·31

TABLE 7 — AVERAGE NUMBER OF LEAVES PER PRIMARY

Tipping height	Number of leaves
5 cm	2·73
10 ,,	3·78
15 ,,	5·00
20 ,,	5·80
25 ,,	6·94
30 ,,	7·60
35 ,,	8·52

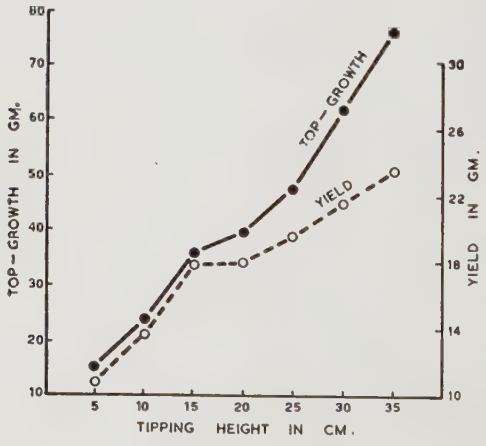

Fig. 2 — Variation of plucking weight (C) and top-growth (C+P) with tipping height.

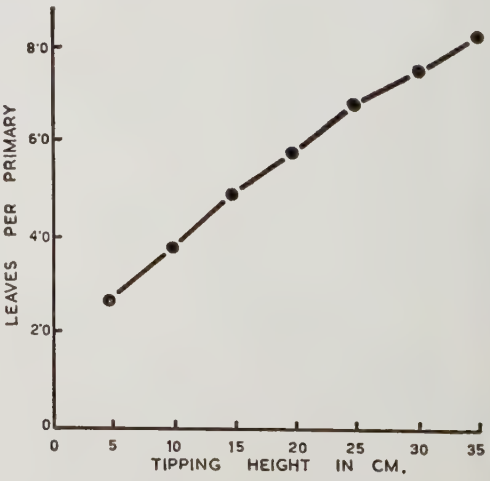

Fig. 3 — The average number of leaves on a primary at different tipping heights.

Yield increases in proportion to the number of leaves per primary up to a tipping height of 15 cm. Above this there is either no increase or, with sufficiently high tipping, a loss of yield (Fig. 1). Reference to Table 7 suggests that less than four leaves per primary is insufficient to support maximum yield and more than five leaves seems uneconomic.

The number of leaves per primary has a greater influence on total top-growth than on yield alone (Fig. 1). The difference is due to pruning weight, and this is important because it measures increments to the permanent bush-frame which has a positive influence on the continued efficiency of the bush as a source of plucked shoots.

A proportionality between leaf frequency and top-growth (Fig. 1) shows distinct breaks at 15 cm and again at 30 cm tipping heights. The curve for top-growth shows (vide Table 7) that six (5·8) leaves per primary is no better than five leaves. With more than six leaves there is an increase of top-growth up to about eight leaves (7·6), but nine leaves (8·5) is associated with less top-growth than eight leaves. This is another aspect of phasic regeneration of growth to which reference has already been made, and it strongly suggests that, in addition to the number of leaves, other factors occurring periodically along the length of the stem also have a significant influence on regeneration. This matter will be discussed in the next section.

Phasic Distribution of Leaves — Under the conditions of pruning exemplified by the experimental clone, it is common for the growing apex of a primary shoot to form a resting bud after unfolding five normal foliage leaves. The resting bud terminates the *first flush* of a primary shoot. The number of leaves on the first flush is comparatively independent of large variations in soil nutrients (Wight, 1934).

The apices of those first flush shoots, which resume growth in length, eventually form resting buds after unfolding a number of leaves. The resting bud marks the limit of the *second flush.*

Observations carried out on the unplucked bushes of the experimental clone showed modal heights of dormancy for the first and the second flushes to be associated respectively with five and eight leaves per primary (Table 8).

Turning now to the upper curve in Fig. 1, the marked inflexions at 15 cm and 30 cm tipping heights correspond to the modal heights of dormancy of the first and the second flushes. Despite an increase in the number of leaves per primary, top-growth was reduced by tipping just above these modal heights.

The same considerations apply to the corresponding inflexions of the curves in Fig. 2. It will be shown in the next section that the bush-frame also shows a phasic response to tipping height.

Secondary Growth — It is the integration of the activities of the primary shoots that is important. Thus far, these shoots have been integrated in terms of yield and top-growth which are removed from the bush. But an integration in terms of the permanent frame of the bush may be possible. As divisions of the secondary cambium in general are in some way dependent on the apical meristems (McDougal, 1938; Richardson, 1958), the radial growth of the limbs of the bush-frame of tea might be an integration of the activities of innumerable stem apices. Fundamental investigations of radial growth have been largely based on woody plants of the temperate zone where there is a single, well-marked phase of dormancy per year. The radial growth of the tea plant, with repeated phases of apical dormancy is, therefore, of special interest.

TABLE 8 — MEAN AND MODAL HEIGHTS OF DORMANCY FOR THE FIRST AND THE SECOND FLUSHES

	Mean	Mode	
		Observed	Calculated*
First flush	21·8 cm	17·0 cm	15·7 cm (5·0)
Second flush	39·9 cm	34·5 cm	32·8 cm (8·1)

*Barua (1953). The number of leaves is shown in parenthesis

Radial growth of representative branches on the frame of each bush was measured at approximately half the vertical height between the ground and the pruning level. Two mutually opposed diameters on each branch of the frame were measured at marked points in the beginning and at the end of the experiment. Fifteen branches were measured in each treatment. Data, given in Table 9, are illustrated in Fig. 4. Apart from differences in the height of tipping, the treatment of all bushes was identical.

TABLE 9 — INCREASE OF BRANCH DIAMETER

Tipping height	Mean diameter of a branch in cm on 4th March	Per cent increase of branch diameter between 4th March and 19th November	Mean apical activity
5 cm	1·656	4·44	1·54
10 ,,	1·654	4·76	1·85
15 ,,	1·656	6·30	1·82
20 ,,	1·537	6·80	2·04
25 ,,	1·610	6·90	2·04
30 ,,	1·628	7·95	2·08
35 ,,	1·669	9·00	2·38
Not tipped	1·435	9·73	—

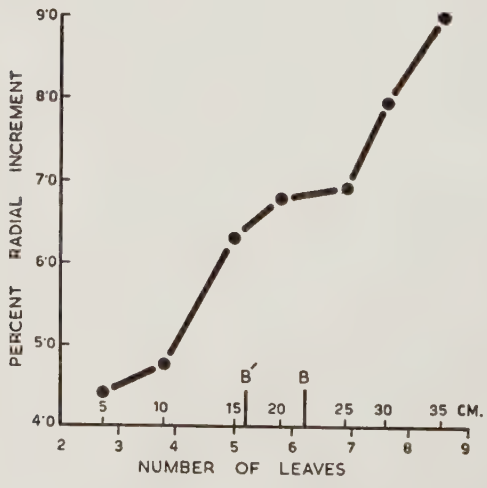

Fig. 4 — Radial growth associated with increasing number of leaves per primary at different tipping heights.

Figure 4, therefore, represents a relation between radial growth and the number of leaves on a single primary shoot. The figure clearly shows that factors additional to the number of leaves influence radial growth.

The mean and the modal heights of dormancy are indicated as B and B′ on the basal scale of leaf sequence in Fig. 4. The figure shows that radial growth increases in proportion to the number of leaves added to the primary stem, both above and below the region of the dormant bud. Additional leaves in the region of dormancy have little or no effect on radial growth.

It would appear from Fig. 4 that the rate of radial growth began to diminish while the shoot apex was still in a growing condition and before a dormant bud was formed. Radial growth ceased with the formation of the dormant bud, and resumed only after a foliage leaf was added to the primary axis above the position of the previously dormant bud. Therefore, it seems that bud-break is necessary for cambial activity. Along these lines of thought, the activity of the cambium should show some correlation with the activity of the shoot apices.

Degrees of dormancy at the apex have been defined by a *dormancy index* (Wight & Barua, 1955). Dormancy indices for each tipping height were determined at every plucking round and the yearly averages were worked out from these records. The reciprocal of the dormancy index can be presumed to measure an intensity of processes defined as ' apical activity '. The mean apical activity so defined, associated with each tipping level, is shown in Table 9. There is a considerable similarity between the trends of radial growth and apical activity along tipping heights, the correlation coefficient between the two series being 0·92, significant at 1 per cent level of probability. The per cent increments of radial growth (Table 9) could, therefore, be considered a natural and probably very accurate estimate of the integrated activity of innumerable stem apices. Possibly more important is the fact that radial growth can be regarded as a function of apical activity as defined (Fig. 5). It will be appreciated that the definition is simple,

metrical and easily applied to large scale work in the field.

Discussion

Top-growth increases with increasing number of leaves on the primary shoots; so does radial growth. This emphasises the importance of the leaves left permanently on the primary shoots. The importance of the permanent leaves is further enhanced by the observation that photosynthetic efficiency develops gradually in a tea leaf and that a leaf does not become fully efficient until it has reached approximately half its maximal size (Barua, 1953). A very large fraction of the young leaves that are removed as pluckings would not reach that stage of development.

The total yield of shoots obtained from a bush is the product of yield per primary and the number of primaries. It has been shown that the number of primaries steadily decreases, and yield per primary increases as the tipping height is progressively raised. However, the rate of yield increase per primary slows down above the first horizon of dormancy (Fig. 2). The product of these mutually opposing trends should come to a maximum at some point intermediate between the two extreme heights of tipping. The inflexion of the curve in Fig. 2 causes this point to coincide with the modal height of dormancy for the first flush.

Secondary growth of the primaries shows a steady and significant increase with increasing height of tipping (Table 10). This gives added weight for a rise in height and the proportionality between top-growth and tipping height is thus maintained. There are, however, marked points of inflexion in the top-growth curve of Fig. 1, associated with horizons of dormancy. These inflexions cannot be considered accidental, because radial growth much lower down the caulome, which was shown to be an integration of activities of numerous stem apices, also shows a pronounced inflexion about the first horizon of dormancy. Since all apices in a given location tend to be synchronous in their phases of dormancy or activity (Wight & Barua, 1955), it is, therefore, considered that the observed inflexions are primarily determined by a rhythm inherent in the stem apices, their position in space being incidental to the inherent rhythm.

The height of dormancy is the fundamental thing, not the absolute height. Under conditions of the experiment, a tipping height of 15 cm, corresponding to the first modal height of dormancy, produced the maximum yield. The optimum tipping height for a different clone or for the same clone under a different set of conditions could have been above or below 15 cm. The principle appears to be that tipping much above or much below the level of mean dormancy can be noticeably less efficient than tipping at that level.

Our observations were based on vigorous plants. We would be far from sug-

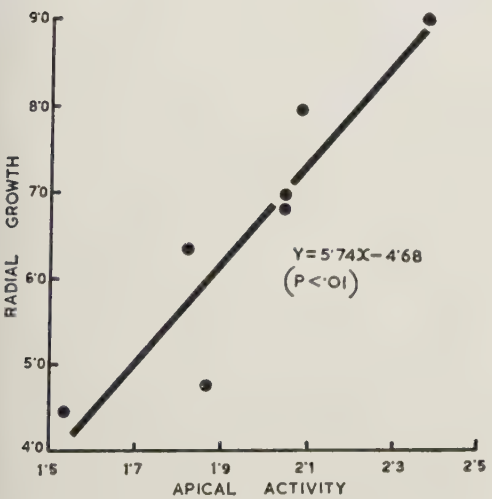

FIG. 5 — Regression of radial growth on apical activity.

TABLE 10 — MEAN DIAMETER OF CURRENT SEASON'S STICKS IN CM

TIPPING HEIGHT	MEAN DIAMETER
5 cm	0·472
10 ,,	0·508
15 ,,	0·582
20 ,,	0·592
25 ,,	0·625
30 ,,	0·665
35 ,,	0·693
Not tipped	0·632

L.S.D. at P=0·05: 0·030 cm.

gesting that impoverished bushes (of which far too many exist) with a dormant horizon which might be about 10 cm should be tipped at that level. Such bushes should probably be tipped at the dormancy horizon of the second flush, if one has in mind the continuance of the bushes as a capital investment.

One might note in the above connection that a circulation of nutrients, by return of prunings to the top soil, is an important part of the system of cultivation followed in north-east India. The importance of the pruning litter in maintaining productivity of the tea bush has been demonstrated by field trials (Cooper, 1939). The prunings probably provide, in readily available form, many of the nutrients needed in the next year.

Finally, a tea bush is required to maintain productivity for about 50 years. Continued efficiency of the bush depends on the growth of its limbs. In this context, radial growth is an important asset. If one considers radial growth, then the mean height of dormancy appears to be the correct tipping level. An average tipping height of 20 cm has long been a practical recommendation. In view of the above considerations, this recommendation appears sound in principle.

Summary

The system of pruning, tipping and plucking of the tea bush, as practised in north-east India, is described. Bushes belonging to a clone representative of the tea grown in the plains were tipped at heights varying from 5 cm to 35 cm in steps of 5 cm. The average number of leaves per primary increased, and the number of primaries on a bush decreased in proportion to the rise of the tipping level. Tipping at the first modal height of dormancy, with an average of 5 leaves per primary below the tipping level, produced the maximum yield.

Proportionality between top-growth and leaf frequency was disturbed at the first and the second horizons of dormancy, thereby indicating influence of factors additional to the number of leaves.

Radial growth increased in proportion to the number of leaves above and below the first region of dormancy: additional leaves in this region had little or no effect. The suggested dependence of cambial growth on the activity of stem apices was demonstrated by a correlation coefficient of 0·92 between radial growth and apical activity. It was concluded that radial growth is an integration of numerous stem apices, and conversely that apical activity as defined is indicative of radial growth.

The authors are indebted to Dr E. K. Woodford, now the Director, Unit of Experimental Agronomy, University of Oxford, for initiating the investigation; to the Director, Tocklai Experimental Station, and the Indian Tea Association for permission to publish.

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STUDIES IN THE ANNONACEAE — I. MICROSPOROGENESIS IN *CANANGA ODORATA* AND *MILIUSA WIGHTIANA*

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Introduction

Schnarf (1931) has summarized the embryological data of the Annonaceae available up to 1931. Subsequent contributors are Juliano (1935), Locke (1936), Asana & Adatia (1947), Corner (1949) and Sastri (1957). Pertinent information from the studies of these authors will be taken up for discussion at appropriate places.

Material and Methods

Material for the present study was collected from plants cultivated in Madras, and fixed in FAA. Customary methods of serial sectioning were adopted. Staining in phenolic Haematoxylin with Fast green, Orange G or Erythrosin as counter stain proved satisfactory.

Observations

Cananga odorata (Lam) Hook. f. & Thoms. — The stamens arise as finger-like protuberances from the almost flat thalamus. Immediately after emergence they consist of a mass of undifferentiated meristematic cells. Differentiation commences with the isolation of two groups of deeply staining cells along the adaxial side towards the basal portion of each elongating primordium. In transections of the flower at this stage those primordia which are cut through the extreme apices appear circular in outline with a homogeneous mass of cells (Fig. 52), whereas those cut through the middle show the two groups of deeply staining cells (Figs. 53, 54). The transectional appearance of the staminal primordia at this stage has a remarkable resemblance with the carpel primordia in which the marginal foliar meristems is just differentiated (Fig. 54). In the basal portion of the staminal primordia also differentiation does not extend up to the extreme base but stops short just above the junction of the primordium to the thalamus (Fig. 1). This basal undifferentiated portion matures as the short filament of the stamen (Figs. 2-5). Thus the stamen soon attains its mature configuration into a sterile apex, a fertile theca-bearing region, and a short filament (Figs. 1, 2). In other words, the several regions of the mature stamen become blocked out at a very early stage and they retain their distinctness up to the end, the subsequent growth taking place essentially by intercalary divisions and cell enlargement in the respective regions.

The undifferentiated apical portion develops into the sterile conical prolongation beyond the anther sacs in the mature stamen. In contrast to the basal fertile portion, the sterile apex elongates rapidly and attains several times the length of the former (Figs. 1, 2). Before active development commences in the fertile portion, the sterile apex becomes almost fully developed and its epidermal cells towards the tip become thick-walled and papillose (Figs. 3-6).

Following early differentiation, the theca-bearing region becomes four-angled (in transectional view). Each group of deeply staining cells becomes split into two by the parenchymatization of the intervening one or two layers of cells (Fig. 55). Simultaneously a single row of hypodermal cells (Fig. 7) becomes differentiated as the primary archesporium at the four corners. Each archesporial cell divides periclinally into an outer primary

FIGS. 1-15 — *Cananga odorata* (*E*, epidermis; *P*, parietal layer; *T*, tapetum; *W*, wall layers). Figs. 1-5. Median l.s. of stamens showing successive stages in development. × 44. Fig. 6. L.s. of the tip of the connective. × 175. Fig. 7. L.s. of a young anther locule showing the uniseriate row of archesporial cells. × 520. Figs. 8-10. T.s. of stamens showing successive stages in the development of the wall layers (only one locule shown). × 520. Figs. 11,12. L.s. of anther locule showing sterilization of sporogenous cells and formation of sterile septa. Fig. 11. × 365. Fig. 12. × 175. Fig. 13. Portion of l.s. of a transversely partitioned anther locule (microspores not shown). × 175. Fig. 14. Portion of t.s. of mature anther, × 115. Fig. 15. Germinating pollen grain. × 235.

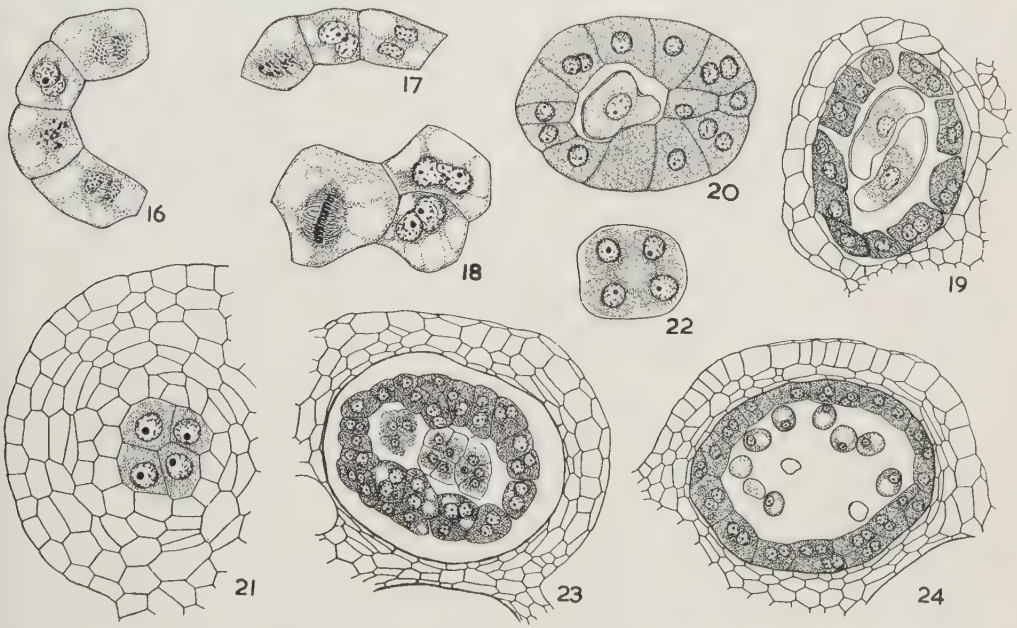
parietal cell and an inner primary sporogenous cell (Fig. 8). The primary parietal cell undergoes two or three periclinal divisions so that three or four wall layers are formed. Of these the outermost functions as the endothecium and the innermost as the tapetum while the remaining 2 or 3 constitute the middle layers (Figs. 8-10).

The epidermis and the middle layers disorganize during later stages. The endothecium develops the characteristic fibrous thickenings. Two peripheral layers of cells in the connective region become thick-walled in continuation of the epidermis which also attains a similar modification. Thus the connective region becomes clearly marked out from the pairs of thecae (Fig. 14).

The tapetum is of the secretory type (Fig. 19) and the cells become binucleate shortly before the commencement of meiosis in the microspore mother cells (Figs. 12, 16). Sometimes the nuclei undergo another division (Fig. 17) so that the cells become four-nucleate. Occasionally the two spindles fuse at metaphase to produce two tetraploid nuclei (Fig. 18). As may be expected, these are slightly larger than the diploid nuclei. The tapetum begins to degenerate after the formation of the microspores.

In a few anther thecae the tapetum exhibits a deviation from the normal behaviour. After the formation of the microspores, the tapetal cells enlarge and elongate radially to occupy almost the entire cavity of the theca. The cells become uniformly filled with alveolar cytoplasm and are binucleate (Figs. 20, 56). In such thecae the microspores degenerate without further development.

The archesporial cells function directly as microspore mother cells (Fig. 49). In some thecae, however, the microspore mother cells are interrupted by sterile transverse partitions (Figs. 12, 57). This condition appears to result in two ways:



FIGS. 16-24 — Figs. 16-20, *Cananga odorata*. Figs. 16-18. Behaviour of nuclei of tapetal cells (for explanation *see* text). × 520. Fig. 19. T.s. of anther locule showing tapetum after formation of microspores. × 230. Fig. 20. Abnormal tapetum. × 230. Figs. 21-24. *Miliusa wightiana*. Fig. 21. T.s. of an anther locule showing massive wall and sporogenous tissue. × 520. Fig. 22. Early stage in cytokinesis of microspore mother cell. × 520. Figs. 23, 24. T.s. of anther locules showing two successive stages in development of microspores. × 230.

(1) Some of the sporogenous cells in the row divide by a periclinal wall (Figs. 11, 61a); the derivatives soon become sterilized (Figs. 61-63) and look like tapetal cells in regard to their histological behaviour (Figs. 12, 57); (2) the archesporium itself arises as a discontinuous row so that units of varying number of sporogenous cells become isolated in smaller compartments (Fig. 13). The latter condition, however, is comparatively rare.

The first indication of meiosis in the microspore mother cell is a change in the pattern of the cytoplasm of the cell. The uniformly distributed cytoplasm aggregates as a dense homogenous mass just around the centrally placed nucleus, but thins out in the form of fine radiating strands towards the periphery of the cell (Fig. 25). Simultaneously, the nucleus begins to exhibit the usual changes of the meiotic prophase. Eight chromosomes were observed in polar views of the metaphase plate (Fig. 26).

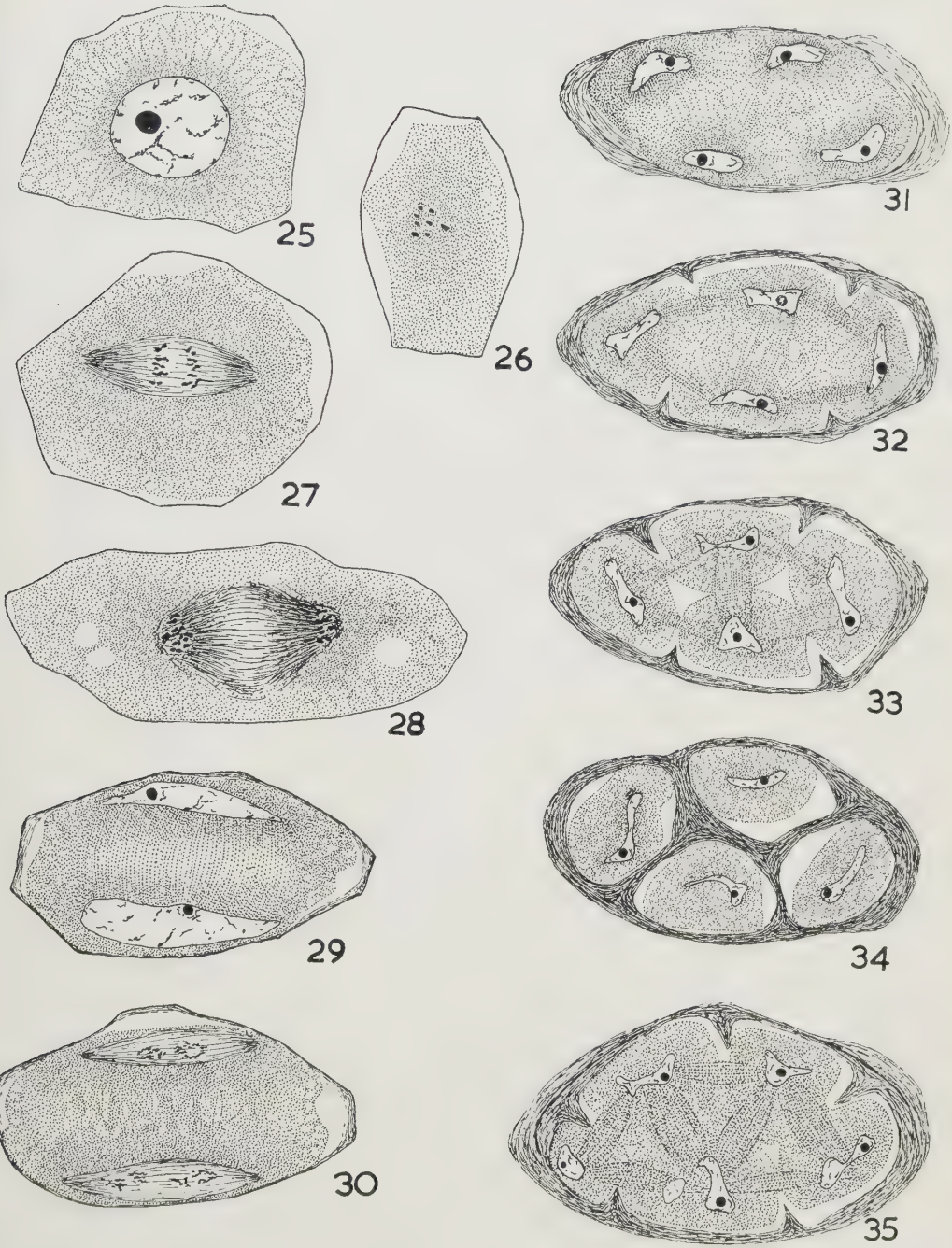
The spindle fibres of the first division disappear without forming a cell plate (Figs. 27, 28). Simultaneously with this and the organization of the energic nuclei there is a thinning out of the cytoplasm along the equatorial region. The nuclei become pushed apart towards the periphery, become spindle shaped and get somewhat hypertrophied (Fig. 29). In the second division the orientation of the spindles is usually such as to give rise to a tetragonal microspore tetrad (Figs. 30, 31). In a few instances, however, an isobilateral or decussate arrangement is also met with.

By the time the first meiosis nears completion, the microspore mother cell wall begins to swell (Fig. 29). The swelling commences first at places where the cytoplasm has contracted away from the wall. Gradually the remaining portion of the wall also undergoes similar modification and by the time the microspore nuclei are well organized, a major portion of the wall becomes uniformly swollen (Fig. 32).

The spindles of the second meiotic division disappear completely before the commencement of cytokinesis and the nuclei arrange themselves near the periphery (Fig. 31). The first step in cytokinesis is a relative aggregation of the cytoplasm around each nucleus and a thinning out of the same in the central portions of the cell (Fig. 31). As a result, the cytoplasm gradually assumes a spindle-like pattern in between the nuclei (Figs. 31-33). This is only a consequence of fine vacuolation of the cytoplasm in the thinned out portions between the nuclei and has no similarity with the fibrillar spindle formed during nuclear divisions. Neither is this spindle-like pattern instrumental in bringing about cytokinesis in contrast to the true phragmoplast.

With the progress of cytoplasmic aggregation around the nuclei, conspicuous cleavage furrows start at the periphery in between the nuclei (Figs. 32, 58). The swollen mother-cell-wall-material invades these clefts in the form of wedges (Figs. 32, 58). The cytoplasmic cleavages and the corresponding wedges of wall-material advance centripetally while the cytoplasm in the centre thins out markedly in the two triangular portions enclosed by the five spindle-like structures connecting the four nuclei (Fig. 32). This thinning out causes the formation of conspicuous vacuoles which gradually fuse to produce two large triangular spaces bounded by spindle-like structures (Figs. 33, 59). The mother-cell-wall-material which wedges into the peripheral furrows is absent in these central cleavages (Figs. 33, 59).

The peripheral furrows continue to advance centripetally together with the wall-material and the central vacuoles enlarge by extension of the angles in a centrifugal manner. As a result, each of the spindle-like cytoplasmic connections extending between neighbouring microspore nuclei becomes narrowed down in the middle. Finally, the connections become severed so as to complete the quadruplication of the microspore mother cell (Figs. 34, 60). The peripheral wedges of the mother-cell-wall-material advance and meet in the centre so that the microspores become surrounded by the common wall-material (Figs. 34, 60). In certain cases, however, the swollen mother-cell-wall-material totally disappears even before the completion of cytokinesis. But, the quadruplication proceeds normally as described above so that four, almost naked, microspores

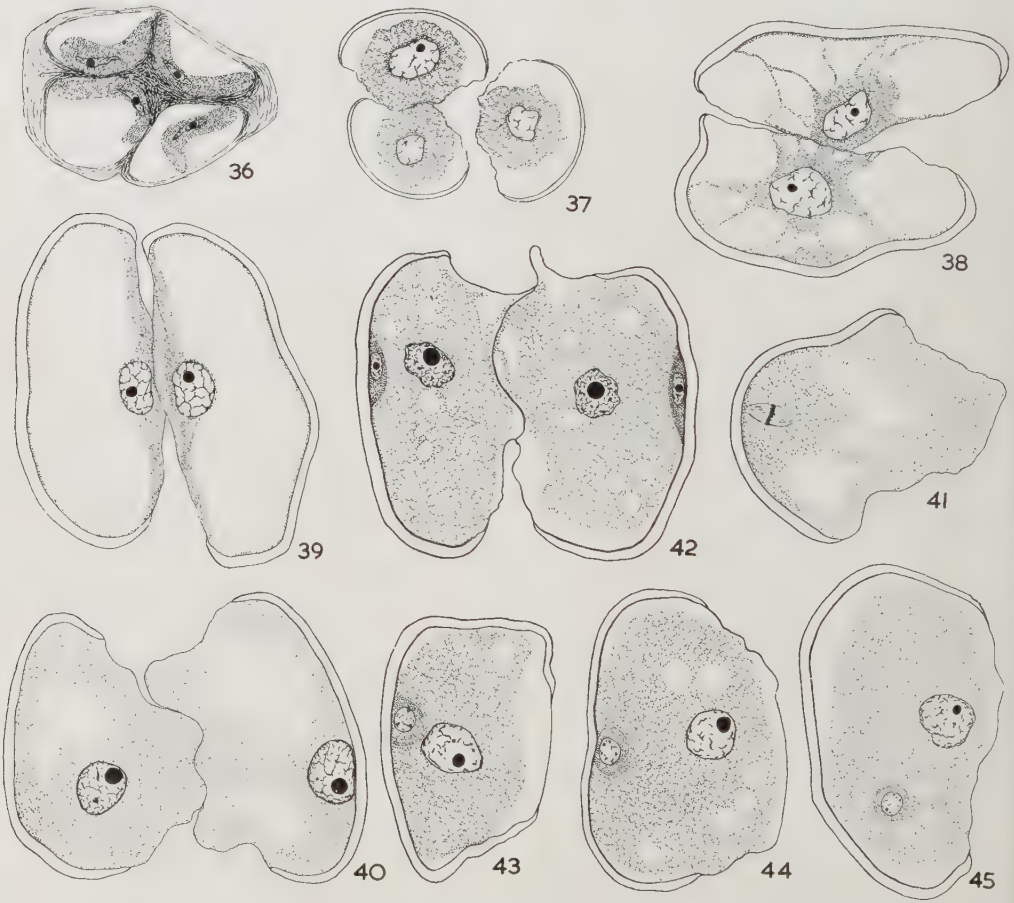


FIGS. 25-35 - *Cananga odorata*, meiosis in microspore mother cells. Fig. 25. Mother cell just before commencement of meiosis. Fig. 26. Metaphase plate of first division showing 8 chromosomes. Fig. 27. Equatorial view of first division spindle. Fig. 28. Late anaphase of first division. Fig. 29. Energic stage just before second division. Fig. 30. Anaphase of second division. Fig. 31. Second division completed; note vacuolation and cytoplasmic pattern. Figs. 32-34. Stages in cytokinesis (for explanation *see* text). Fig. 35. Abnormal mother cell with five nuclei. Fig. 26. × 600; others. × 780.

are formed (Fig. 61). This behaviour appears to be an abnormality.

After quadripartition the mother-cell-wall-material gradually disappears, first from the distal and later from the proximal faces of the microspores (Fig. 36). Corresponding with the disappearance of the mother-cell-wall-material, a true wall becomes differentiated around each microspore (Fig. 37).

The cytoplasm of the microspore exhibits a series of changes. Even before the complete disappearance of the mother-cell-wall-material, the protoplasts of individual microspores of a tetrad contract towards the proximal side in the form of a crescent. The nucleus also becomes somewhat elongated and spindle-shaped (Fig. 36). After the development of the spore wall, the cytoplasm as well as the nucleus assume a spherical contour (Fig. 37). During subsequent stages, the cytoplasm does not increase in such a proportion as to fill the entire lumen of the rapidly widening microspore. Hence, there is an increasing vacuolation and when the microspore has enlarged to its maximum size, the cytoplasm forms only a thin



FIGS. 36-45 — *Cananga odorata*, development of the pollen grain. Fig. 36. Microspore tetrad showing the contraction of the protoplasm towards the proximal side. Fig. 37. Young microspores at the time of separation from each other. Figs. 38-40. Lateral views of microspore pairs showing changes in the cytoplasmic pattern during development. Fig. 41. First division of microspore nucleus. Fig. 42. Two-celled pollen grains soon after division of the microspore nucleus. Figs. 43-45. Successive stages in the behaviour of the generative cell. All. × 520.

peripheral lining layer against the wall (Figs. 38, 39). The nucleus lies at the proximal pole.

As soon as the microspore attains its full size, the quantity of cytoplasm increases enormously so as to fill the lumen completely. During this process the nucleus also shifts its position from the proximal pole and presses itself against the distal pole (Fig. 40).

The first division of the microspore nucleus is accomplished *in situ* (Fig. 41), resulting in the formation of a generative cell and a vegetative cell. The generative cell which is cut off against the distal wall is small, lenticular and densely basichromatic (Fig. 42). However, it gradually assumes an ovoid or spherical contour, becomes separated from the distal wall and comes to lie in the cytoplasm of the vegetative cell (Figs. 43-45). Even though the generative cell loses much of its initial basichromaticity, its thin cytoplasmic sheath is clearly distinguishable from the alveolar cytoplasm of the vegetative cell (Figs. 42-45).

The wall of the young microspore is relatively thicker at the distal hemisphere than at the proximal where it is highly tenuous (Fig. 37). The same relationship is maintained throughout further development and also in the mature pollen grains (Figs. 38-45) which remain attached in pairs by their thin proximal walls (Figs. 40, 42). The pollen grains are shed at the two-celled stage.

The behaviour of the anthers at the time of dehiscence has been described in detail by Periasamy (1954). Soon after anthesis, the androecium as a whole becomes separated from the thalamus dragging the stigmatic heads with it. Because of this peculiar behaviour the chances of fertilization are minimized resulting in a low fruit set in and around Madras.

In view of the suspicions raised by Bailey & Nast (1943), as to the position of the germinal area of the pollen grain in the Annonaceae, attempts were made to study the development of the pollen tube in *Cananga odorata*. Much difficulty was encountered in successfully germinating the pollen grains either in distilled water or in sugar solutions. But it was possible to obtain the early stages by dusting mature pollen grains over the stigma which is then decapitated and placed in a moist chamber. All the pollen grains germinated put forth pollen tubes from the thinner proximal surface (Fig. 15).

In two instances it was seen that a single microspore mother cell had given rise to five microspore nuclei instead of four (Fig. 35).

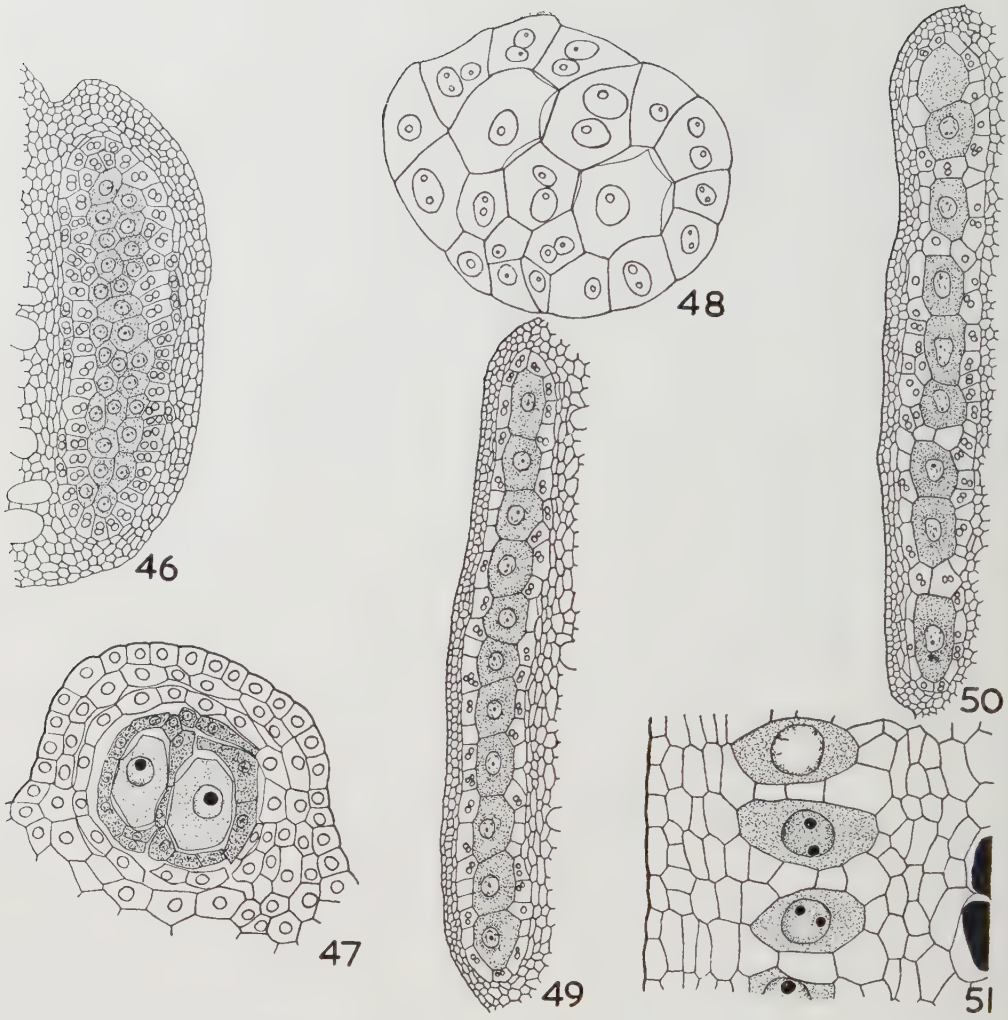
Miliusa wightiana Hook. f. & Thoms. — This plant differs from *Cananga odorata* in the following points.

The sporogenous tissue is massive and the anther wall is more than four layers in thickness (Fig. 21). Sterile transverse partitions do not occur in any of the thecae. The apical prolongation of the connective beyond the anther sacs is flat and hoodlike.

The cytoplasmic changes which accompany meiosis and cytokinesis of the microspore mother cell are essentially similar to those in *Cananga odorata*. However, the swelling of the mother cell wall is much delayed in *Miliusa wightiana* so that no wedges of wall-material follow the peripheral cleavages during the early stages of cytokinesis (Fig. 22). The individual microspores become surrounded all at once by the mother-cell-wall-material after the complete separation of the protoplasts of the tetrad. The microspores separate from each other and lie free in the anther sac (Fig. 24). The pollen grains are monocolpate with an unornamented uniformly thick exine.

Discussion

Stamen — The protrusion of the connective beyond the anther is a characteristic feature of the Annonaceae. Parkin (1951) considers the protrusion as representing the sterilized terminal part of the ancestral microsporophyll in the preangiospermous stock, and rejects the possibility of its being a new outgrowth upon an initially terminal microsporangium as envisaged by Thomas (1932, 1936) and Wilson (1937). In *Cananga odorata* the terminal portion of the staminal primordium becomes sterilized at a very early stage in ontogeny and develops into the protruded connective. In the



FIGS. 46-51 — Sections of microsporangium illustrating progressive reduction of the sporogenous tissue in the Annonaceae. Fig. 46. *Miliusa wightiana*, l.s. of microsporangium showing massive sporogenous tissue. Figs. 47-48. *Asimina triloba*, t.s. of microsporangium showing two rows fo microspore mother cells and intervening sterile partition (after Herms, 1907). Fig. 49. *Cananga odorata*, l.s. of microsporangium showing uniseriate microspore mother cells. Fig. 50. Same as Fig. 49 showing uniseriate microspore mother cells interrupted by sterile partitions. Fig. 51. *Annona muricata*, l.s. of microsporangium showing regularly alternating uniseriate microspore mother cells and sterile partitions (after Juliano, 1935).

basal portion, the meristematic regions from which the sporogenous cells differentiate, are confined to the two lateral sides towards the adaxial face and simulate the marginal meristem of the foliar appendage as well as that of the conduplicate carpel (Periasamy & Swamy, 1956). These ontogenetic sequences support the foliar concept of the stamen rather than the views expressed by Thomas and Wilson.

SPOROGENOUS TISSUE — With regard to the sporogenous tissue, *Artabotrys odoratissimus* (Asana & Adatia, 1947) and *Miliusa wightiana* represent one group with a massive sporogenous tissue, while

Annona muricata, A. squamosa (Juliano, 1935), *Asimina triloba* (Herms, 1907; Locke, 1936), *Monodora, Xylopia* (LeComte, 1896) and *Cananga odorata* represent the other group with a uniseriate sporogenous tissue in each theca. In *Artabotrys odoratissimus* as well as in *Cananga odorata* the sporogenous tissue consists of only two groups of cells in the beginning, but later becomes separated into four.

The genera with a uniseriate sporogenous tissue invariably show sterile partitions between the microspore mother cells. Schnarf (1931) assumes the absence of sterile partitions in *Cananga odorata* on the basis of the accounts of Oes (1914) and Stark (unpublished), but the present investigation shows the frequent occurrence of sterile septa varying from one to four in a number of thecae. In *Annona, Monodora* and *Xylopia* the sterile septa regularly alternate with the microspore mother cells (Schnarf, 1931, Juliano, 1935). *Asimina triloba* (Herms, 1907; Locke, 1936) with only two rows of microspore mother cells separated by a sterile longitudinal partition in each theca, appears to represent an intermediate condition between the massive and the uniseriate sporogenous tissue. Thus, in the Annonaceae there seems to be a progressive reduction of the massive sporogenous tissue as seen in *Artabotrys* and *Miliusa* (Fig. 46) to the condition found in *Annona, Monodora* and *Xylopia* (Fig. 51), through stages represented by *Asimina* (Figs. 47, 48) and *Cananga* (Figs. 49, 50).

In *Cananga odorata* the sterile septa are in most instances transformed cells of the sporogenous tissue itself and behave exactly like the cells of the tapetum. Herms (1907) and Juliano (1935) also record the tapetal behaviour of the sterile septa in *Asimina* and *Annona.*

Tapetum — A binucleate secretory tapetum appears to be characteristic of the Annonaceae (Wunderlich, 1954). In *Cananga odorata,* the two nuclei frequently divide again to give rise to a four-nucleate condition or to a secondary bi-nucleate condition by the fusion of the spindles.

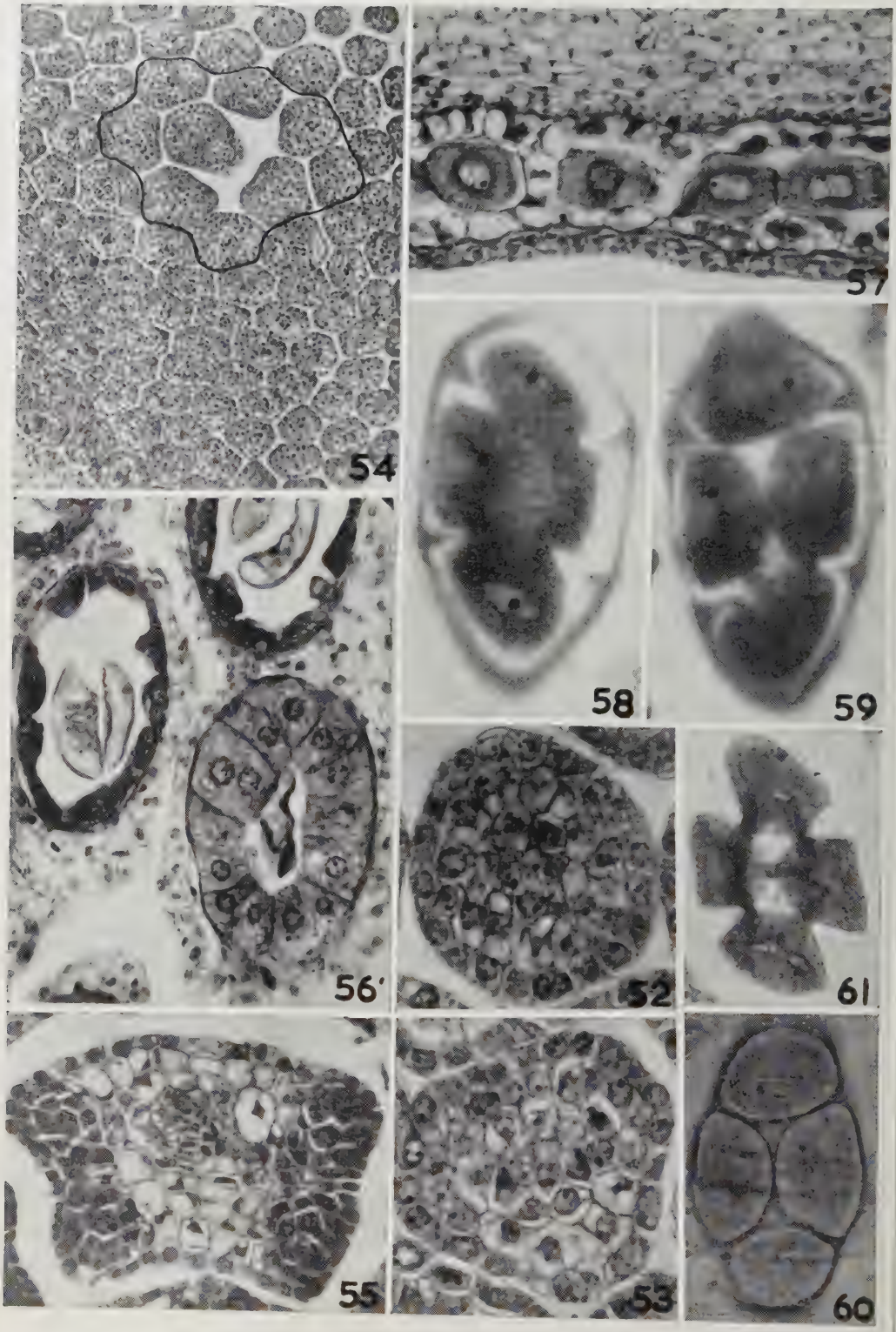
Juliano (1935) reports the formation of a periplasmodium in *Annona,* but without any convincing illustrations. Similarly, without evidence to support, Asana & Adatia (1947) report the occurrence of a periplasmodium in *Artabotrys odoratissimus, Polyalthia, Annona reticulata, A. muricata, A. cherimolia, Unona* and *Cananga.* Since the present investigation shows only a secretory tapetum in *Cananga* and *Miliusa,* it may be suggested that what these authors have seen is not a true periplasmodium, but probably a phenomenon similar to that described in *Austrobaileya* (Bailey & Swamy, 1949) and also in some members of the Lauraceae (V. P. Krishnan, unpublished).

In some of the anther thecae of *Cananga* there is an abnormal enlargement of the tapetal cells with an attendant degeneration of the microspores surrounded by them. Wunderlich (1954) records similar occurrences in a number of unrelated angiosperms.

Reduction division — There is a formation of perikaryoplasma (Lawson, 1898) in the microspore mother cells of *Cananga odorata* and *Miliusa wightiana,* just before reduction division.

The type of division of the microspore mother cell in the Annonaceae is rather controversial. The successive type has been reported in *Cananga odorata* (Oes, 1914), *Annona* (Juliano, 1935), *Uvaria kirkii* and *Annona reticulata* (Sastri, 1957) (*see* also Schürhoff, 1926). On the other hand, a simultaneous type has been reported in *Annona* (Samuelsson, 1914), *Polyalthia* and *Saccopetalum* (Sastri, 1937) (*see* also Schnarf, 1931). Locke (1936) designates the method in *Asimina triloba* as successive constriction. The present investigation shows beyond any doubt a simultaneous division in *Cananga odorata* and *Miliusa wightiana.* Sastri (1957) considers these differences within the family as representing a transition series from the successive to the simultaneous type through genera like *Annona* and *Saccopetalum.*

After discussing the two types of divisions, Schnarf (1929) concludes that only cytokinesis by cell plate formation should be considered as successive while cytokinesis by constriction should be designated as simultaneous division; and

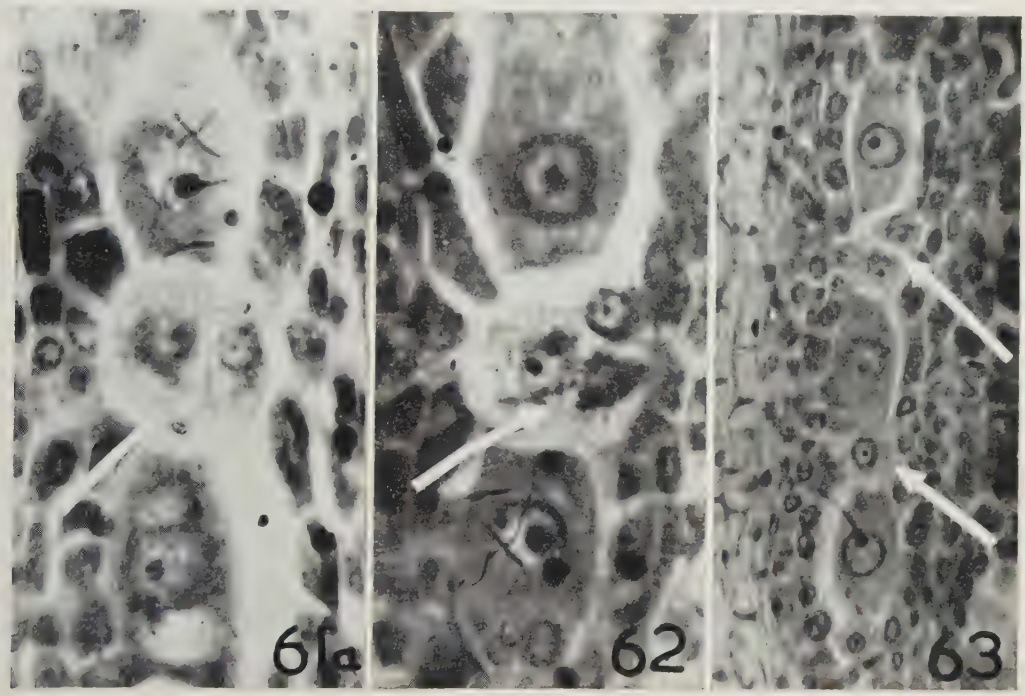


FIGS. 52-61.

that even when constriction takes place in two steps as in *Annona, Asimina,* etc., it should be designated as a modified simultaneous and not the successive type. The remarks of Farr (1916) and Maheshwari (1950) also point to the fact that the terms simultaneous and successive do not signify merely a temporal relationship, but designate two cytological processes with a number of more important differences between them. Since in all the investigated plants of the Annonaceae cytokinesis takes place only by constriction, the weight of evidence is in favour of considering the method of division as belonging to a simultaneous or to a modified simultaneous type and *not* to the successive type.

MICROSPORE — In *Cananga odorata,* the generative cell is cut off against the distal pole (cf. Juliano, 1935, on *Annona*).

The lenticular generative cell, which has a clear cytoplasmic sheath, rounds off and becomes embedded in the cytoplasm of the vegetative cell in both *Miliusa* and *Cananga.* According to Juliano (1935),



FIGS. 61a-63 — *Cananga odorata.* Stages in sterilization (pointed by arrows) of sporogenous cells to form sterile septa.

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FIGS. 52-61 — *Cananga odorata.* Fig. 52. T.s. at the apical region of young staminal primordium. Fig. 53. Same at the middle region. Fig. 54. T.s. of young flower bud just after commencement of differentiation in the primordia of the carpels and stamens. The line encloses carpel primordia. Fig. 55. T.s. of stamen showing differentiation of sporogenous cells. Fig. 56. T.s. of anthers showing abnormal behaviour of the tapetum in one of the locules (lower right hand corner). Fig. 57. L.s. of microsporangium showing sterile transverse partitions between the microspore mother cells. Figs. 58-60. Three successive stages in the cytokinesis of the microspore mother cell. Fig. 61. Microspore mother cell in which the mother cell wall has completely disappeared before completion of cytokinesis.

and Asana & Adatia (1947) in *Annona* and *Artabotrys* respectively, the generative nucleus acquires a cytoplasmic sheath subsequent to its formation, but finally loses it and becomes a naked nucleus imbedded in the cytoplasm of the vegetative cell (*see* Juliano's Fig. 35). In view of the increasing evidence in favour of the occurrence of a generative cell, and not a nucleus, the reports of these authors need confirmation.

The pollen grains of *Cananga odorata* germinate on the proximal side as already hinted by Bailey & Nast (1943).

Summary

The staminal primordia become differentiated into a sterile apical portion, a middle theca-bearing region and a very short filament. Simultaneously with this differentiation apical growth ceases and further development takes place mainly by intercalary division and cell enlargement. The ontogenetic sequences support the concept of the foliar ancestry of stamens and a lateral origin of the microsporangia.

The microsporangium contains a massive sporogenous tissue in *Miliusa* but only a uniseriate row in *Cananga*. Sterile septations resulting from a sterilization of the sporogenous tissue are of frequent occurrence in *Cananga*. The behaviour of the cells, constituting the sterile septations in the microsporangium, is exactly similar to that of the tapetum derived from the parietal tissue in *Cananga* as well as in other plants of the family.

The tapetum is of the secretory type. The cells become binucleate in *Miliusa* and *Cananga*. In *Cananga* there may be a further division of the nuclei resulting in a four-nucleate stage, or a secondary binucleate condition due to the fusion of the spindles. In this plant there is an abnormal development of the tapetum in some of the thecae. The division of the microspore mother cell is of the simultaneous type in *Cananga* and *Miliusa*. The criteria employed to distinguish the successive and simultaneous types of divisions are briefly discussed.

In *Cananga*, the generative cell is always cut off against the distal pole of the microspore. The pollen grains are shed at the two-celled stage. The pollen tube in *Cananga* is put forth from the proximal side of the pollen grain.

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STUDIES OF MORPHOGENESIS IN THE NYMPHAEACEAE — IV. EARLY FLORAL DEVELOPMENT IN SPECIES OF *NUPHAR*

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Introduction

In an earlier investigation of floral morphogenesis in *Nuphar lutea* (L.) Sm. and *N. advena* Ait. it was found that the bract known to be present at the base of the mature peduncle in these species was not a lateral member of the main axis but the first-formed organ of the floral meristem (Cutter, 1957). The flowers of these species, therefore, occur in leaf positions in the genetic spiral, and are not axillary, as considered by earlier workers (*see* Cutter, 1957). In these species the bract is formed in an abaxial (anterior) position on the floral meristem and is homologous with the first sepal in *Nymphaea*. Subsequently the bract develops as a small scale-like organ of rather variable size and form, situated abaxially on the floral peduncle at the level of a slightly contracted region where abscission will subsequently occur. In *Nuphar lutea* two such bracts are occasionally associated with a single peduncle, one being in the normal, abaxial position and the other either opposite to it, in an adaxial position, or lateral to it at a variable angle of divergence (Cutter, 1957, Figs. 8-10).

The bearing of the observations then available on the views of earlier authors and on the nature of the 'bract' in *Nuphar* was discussed in the earlier paper. Further observations on this aspect of floral ontogeny in other species of *Nuphar* are described below. In Britain, two species of *Nuphar* occur, *N. lutea* (L.) Sm. and *N. pumila* (Timm.) DC, and also the hybrid between them, *N.* × *intermedia* Ledeb. The evidence for regarding the latter as a naturally occurring hybrid of *N. lutea* and *N. pumila* has been fully presented and discussed by Heslop-Harrison (1953). In extending the earlier work on floral ontogenesis to *N. pumila* and *N.* × *intermedia*, therefore, an opportunity arises for examining the impact of genetic factors on morphogenesis. Although the comparative morphology of species and the hybrids between them has often been studied, relatively few of these investigations have been concerned with

early stages of development; however, it may well be during these stages that the first manifestations of genetic differences are to be seen.

Materials and Methods

Rhizomes of *N. lutea* were collected from a mere at Tabley, Cheshire; material of *N. pumila* from Aviemore, Inverness-shire, and from Avielochan[1], near Aviemore; and material of *N.* × *intermedia* from Chartners Lough, Northumberland. In addition, some material of *N.* × *intermedia* was obtained from the Royal Botanic Gardens, Kew, through the kindness of the Curator, Mr. W. M. Campbell.

The greater part of the material of *N. pumila* examined was obtained from Avielochan, near the edge of the loch. Heslop-Harrison (1953) has presented evidence which indicates that colonies in this loch have in the past introgressed with *N. lutea*, and are not now genetically pure *N. pumila*. She presents data on the number of vascular bundles in the petioles and peduncles, the number of lateral veins in the leaves, and the number of carpels in true *N. pumila*, ' introgressed ' *N. pumila* from Avielochan, *N.* × *intermedia* and *N. lutea*. These features were accordingly observed in the material examined in the present investigation. There was no apparent difference in the number of vascular bundles in petioles and peduncles of *N. pumila* from the two habitats, but the number of stigmatic rays was slightly higher in the Avielochan material ($11{\cdot}6 \pm 0{\cdot}43$ as against $10{\cdot}25 \pm 0{\cdot}63$). Material of *N. pumila* obtained from Avielochan may be genetically contaminated with *N. lutea* to some extent. Observations on this material have consequently been checked against a more limited amount of material of pure *N. pumila*; no differences have been detected between the two samples in the features to be described in this paper. Material of *N. pumila* illustrated here is from Avielochan, except where otherwise stated.

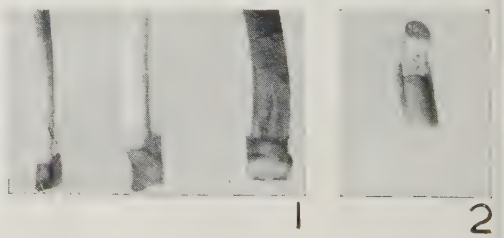
1. On some maps this appears as Avinlochan, and is so termed by Heslop-Harrison (1953); local usage, however, favours Avielochan, and this is presumably correct.

Rhizome tips of the three species were dissected as described in previous papers, and camera lucida drawings of the shoot apex and surrounding primordia were made. Primordia, which are spirally arranged, are called P_1, P_2, P_3, etc., P_1 being the youngest (Snow & Snow, 1931). Material for sectioning was fixed in Craf III (Sass, 1940). Young flowers in various stages of development, and the peduncle bases of older flowers, were sectioned longitudinally in the antero-posterior plane at a thickness of 10 μ. Sections were stained in safranin, orange G and tannic acid with iron alum (Sharman, 1943).

As in the previous paper, in all drawings and photographs of sections of developing flowers the subtending shoot apex is situated towards the right, i.e. the anterior face of the flower is on the left in all figures.

Morphology

At the base of the mature peduncle of *N. lutea*, in an abaxial position, a small scale-like bract from a few mm up to about 1 cm in length is present (Fig. 1, right), as described earlier (Cutter, 1957). The bases of the peduncles of expanded flowers, and of flowers that would expand in the following season, of *N.* × *intermedia* and *N. pumila* were closely examined in order to discover whether or not a comparable structure was present in these species also. In *N. pumila* from Avielochan,

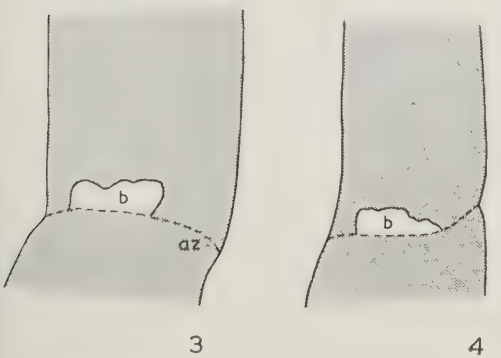


Figs. 1, 2 — Bases of peduncles of *Nuphar* spp., showing the region of insertion of the bract. × ½. Fig. 1. Left to right, *N. pumila* (Aviemore), with no visible bract; *N.* × *intermedia*, with a very small bract, which cannot easily be seen; *N. lutea*, with a conspicuous bract. Fig. 2. *N. pumila*, with a bract of unusually large size.

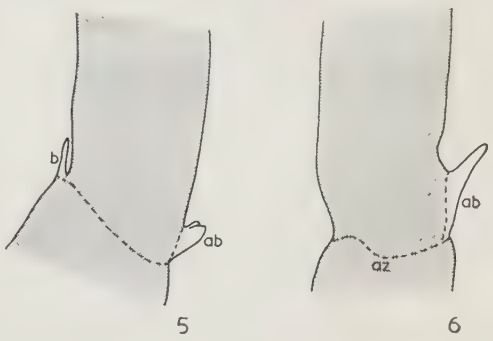
there was no detectable bract in an abaxial position in 83 per cent of the peduncles examined; in the remaining 17 per cent, small bracts were present (Figs. 3, 5) but were sometimes very minute. Indeed, without a careful search they would not have been detected. On one peduncle a much larger bract, about 6 mm long, was observed (Fig. 2). In *N. pumila* from Aviemore no bracts were observed abaxially on the limited amount of material examined (Fig. 1, left). Fairly commonly in material from this source, however, and on 12 per cent of the peduncles obtained from Avielochan, small outgrowths, sometimes scale-like and sometimes rather knobbly, were present at the base of the peduncles in lateral, adaxial or lateral-adaxial positions (Figs. 5-8). These were sometimes adnate to the peduncle over part of their length. In *N.* × *intermedia* from Chartners Lough, bracts in an abaxial position were observed on 10 per cent of the peduncles examined [Figs. 1 (centre), 4]; on 90 per cent, no bract could be seen. In both *N. pumila* and *N.* × *intermedia* peduncles with bracts occurred on the same plant as other peduncles on which bracts could not be seen; sometimes these differed in age by only two plastochrones.

Young developing flowers, about 1 cm long and larger, were also examined for the presence of bracts. None was observed, either in *N. pumila* or *N.* × *intermedia*, on flowers at this stage.

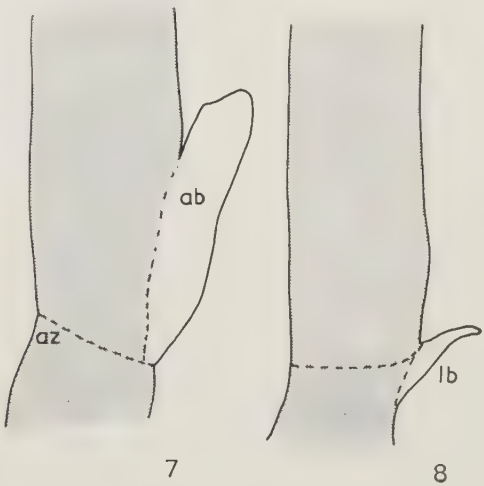
On dissecting inwards until the primordia immediately surrounding the shoot apex could be seen, young flower primordia in various stages of development were



Figs. 3, 4 — Abaxial view of peduncle bases of expanded flowers of *Nuphar* spp. The abscission zone (*az*) is shown as a broken line, the tissue below this being excised from the stock. The bracts (*b*) are shown white. × 3½. Fig. 3. *N. pumila*, with a bract in an abaxial position. Fig. 4. *N.* × *intermedia*, with a bract in an abaxial position.



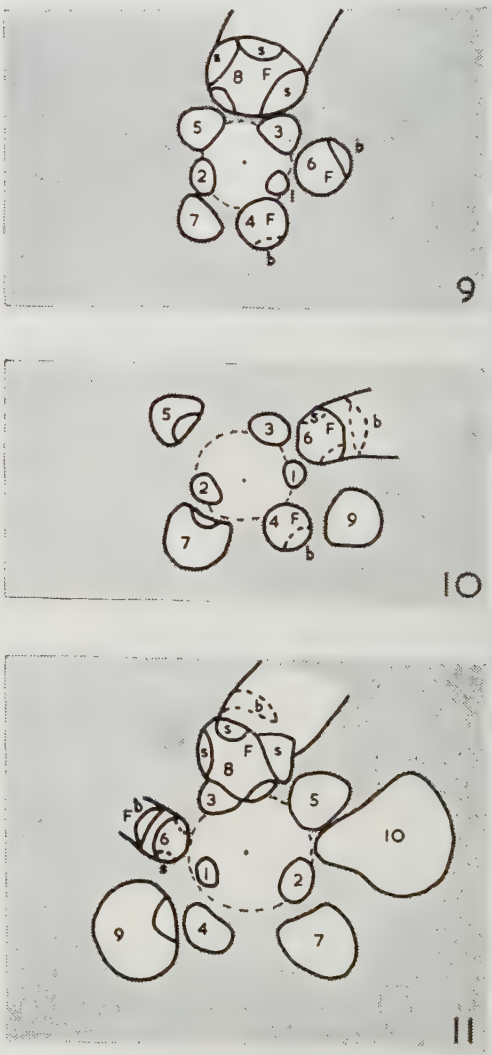
Figs. 5, 6 — Side view of peduncle bases of expanded flowers of *Nuphar pumila* (*ab*, adaxial bract; *az*, abscission zone; *b*, abaxial bract). × 3½. Fig. 5. Both an abaxial bract and an adaxial outgrowth, which is partially adnate to the peduncle, are present. Fig. 6. An adaxial bract, adnate to the peduncle over about half its length, is present, but no bract is visible in the abaxial position (left). From Aviemore.



Figs. 7, 8 — Side view of peduncle bases of *N. pumila* from Aviemore (*ab*, adaxial bract; *az*, abscission zone; *lb*, lateral bract). × 3. Fig. 7. An adaxial bract of considerable size, adnate over about ¾ of its length, is present, but no bract is visible in the abaxial position. Fig. 8. A bract in a more or less lateral position is present, adnate to the peduncle over about half its length, but none is visible in the abaxial position.

carefully examined under a binocular microscope to discover whether bract primordia were present. In flower primordia of *N. lutea* the primordium of the bract can be clearly seen in an abaxial position on primordia older than P_3 (Cutter, 1957). Apices of *N. pumila* and *N.* × *intermedia* were accordingly carefully scrutinized in the light of earlier experience. In *N. pumila*, both from Aviemore and from Avielochan, no bracts could be seen on flowers younger than P_3, but at about this stage there was just a slight indication of a projection abaxially. From external observations alone it could not be decided with certainty whether or not this was an incipient bract primordium. Flower primordia at P_4, P_5 and P_6 (from Avielochan except for one P_5 from Aviemore) showed a distinct bract primordium in the form of an abaxial ridge of tissue of no great height (Fig. 9). At the stage of P_7, flower buds had formed a few sepal primordia, and the bract could sometimes, but not always, be distinguished as a slight ridge. On flower primordia older than P_7 no bract could be distinguished. Presumably it was obscured by the growth of hairs on the peduncle; hair primordia were present on a flower at P_6 all over the abaxial ridge but not on the bract primordium itself.

In *N.* × *intermedia*, bract primordia could be clearly distinguished in an abaxial position on the young flower primordia from the stage of P_4 onwards (Figs. 10, 11). The bract was evident as a collar-like ridge, relatively extensive laterally, but of little vertical height; it was intermediate in size between those of *N. pumila* and *N. lutea*. Hair primordia were again present on the peduncles of flowers older than P_6 up to, but not including, the bract primordium. Sepal arrangement in the flowers of *N. pumila* and *N.* × *intermedia* was similar to that in *N. lutea*, and



FIGS. 9-11.

FIGS. 9-11 — Surface views of rhizome apices of *Nuphar* spp. The approximate extent of the apical meristem is indicated with a broken line; the sub-apical region is stippled. Fig. 9. *N. pumila*. An apex in which P_4, P_6 and P_8 were developing as flowers (*F*). On P_4 an incipient bract primordium (*b*) could just be distinguished, on P_6 it was quite distinct, and on P_8 it had become indistinguishable from the general ridge on the peduncle. P_8 had 4 sepal primordia (*s*), but none was distinguishable with certainty on P_6. Fig. 10. *N.* × *intermedia*. An apex in which P_4 and P_6 were developing as flowers (*F*). Bract primordia (*b*) were evident as a collar-like ridge on both P_4 and P_6, and P_6 had also formed the primordia of the two lateral sepals (*s*). Fig. 11. *N.* × *intermedia* (from Kew). An apex in which P_6 and P_8 were developing as flowers (*F*). A bract (*b*) was evident on both as a ridge of tissue. The meristem has grown on, leaving the bract abaxially on the peduncle; P_8 has formed two, and P_8 four sepal primordia (*s*). All. × 32.

indeed the young flowers of all three species were very similar, though differing somewhat in size.

External observations therefore indicate that in very young flowers of *N. pumila* and *N.* × *intermedia* the primordium of a bract can be detected in an abaxial position, although in *N. pumila* this is so inconspicuous as to be sometimes doubtful; in older flower primordia bracts cannot usually be detected, and they were observed on less than 20 per cent of the peduncles of mature flowers. In *N. lutea* bract development can be followed through all its stages by external observation. In order to follow the stages of bract development in *N. pumila* and *N.* × *intermedia* with greater precision, therefore, young flowers in various stages of development were fixed for longitudinal sectioning in the antero-posterior plane.

Anatomical Observations

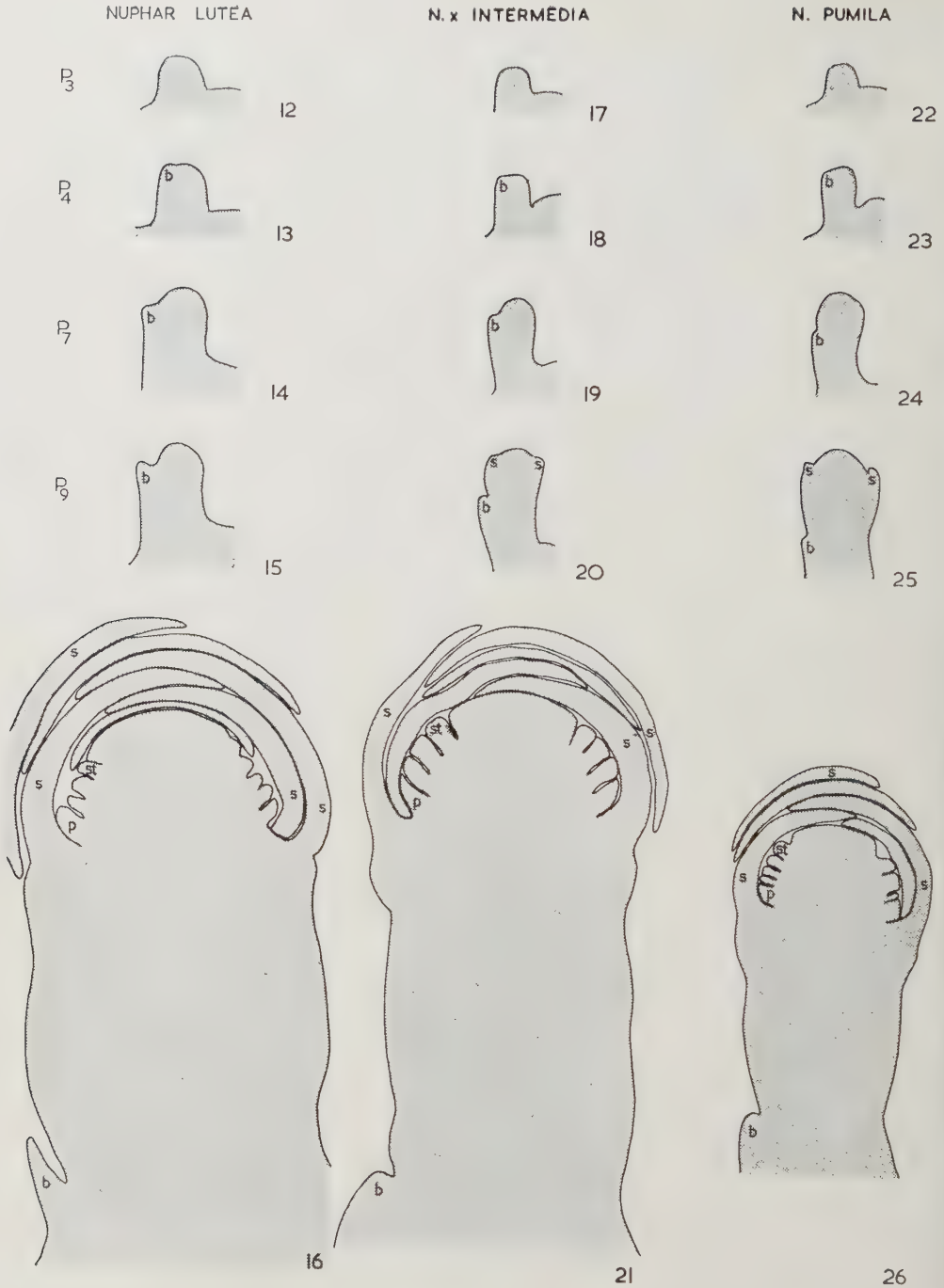
Longitudinal sections of young flower primordia of both *N. pumila* and *N.* × *intermedia* confirmed the invariable presence of a bract primordium in an anterior position. In Figs. 12-26 sections of flowers of *N. lutea*, *N.* × *intermedia* and *N. pumila* of comparable ages as measured in plastochrones are illustrated for comparison. In the previous paper a similar comparison was made between the flowers of *N. lutea*, *N. advena* and *Nymphaea alba*. Within a single species some slight differences may exist between flowers of the same plastochronal age, but these are usually inconsiderable.

Figures 12-26 show that (i) bract inception is comparable in the three species, and occurs at approximately the same age of flower, as measured in plastochrones (Figs. 13, 18, 23); (ii) soon after inception there is a size difference between the bract primordia of the three species, that of *Nuphar lutea* undergoing considerable vertical growth, that of *N.* × *intermedia* much less, and that of *N. pumila* virtually none (Figs. 14, 19, 24); (iii) in *N. pumila* the floral apex grows on at an earlier stage than in *N. lutea*, leaving the bract primordium situated at some distance from it, flowers of *N.* × *intermedia* being intermediate in this respect (Figs. 14, 19, 24); (iv) by about the age of P_9 the bract primordium in *N. pumila* and *N.* × *intermedia* is little more than an inconspicuous ridge of tissue, whereas in *N. lutea* it is a distinct primordium (Figs. 15, 20, 25); (v) at a much later stage of floral ontogenesis, in *N. pumila* and *N.* × *intermedia* this ridge remains only as a slight parenchymatous bulge of a variable degree of development, whereas in *N. lutea* the bract is a clearly evident organ (Figs. 16, 21, 26).

Photographic illustrations of sections of various stages of floral development in *N.* × *intermedia* and *N. pumila* are shown in Figs. 27-34. Of these, Figs. 27, 28, 30, 32 and 34 are the same sections as in Figs. 17, 18, 19, 23 and 24.

During the later stages of floral development in *N. pumila* and *N.* × *intermedia* the amount of development undergone by the bract is rather variable. In sections of some flowers of both species a fairly conspicuous parenchymatous bulge can be observed in an anterior position (Figs. 21, 26, 40); hairs grow out from it. This represents the bract. In other flowers, this projection is much less conspicuous; and in a few it must presumably be conspicuous and undergo further growth, resulting in the presence of a visible bract at maturity, as already described. In these later stages there is apparently little difference in the amount of bract development in *N. pumila* and *N.* × *intermedia*, and within each species this may be rather variable.

In the young flower primordia of *Nuphar* spp. there is a strand of prevascular tissue which traverses the peduncle in an anterior position, just beneath the bract primordium. This is present in all the four species observed, and can usually be detected in flower primordia at about P_4 or P_5, subsequently becoming more conspicuous. In *N. lutea* this prevascular strand is present within the tissues of the bract, whereas in *N. advena* it is not (Cutter, 1957). In Figs. 35-37 the presence of prevascular tissue in the bract primordia of *N. lutea* at various stages of floral development (P_7, petal formation, ovary formation) is shown. Sections of the young flowers of *N.* × *intermedia* and *N. pumila* at comparable stages, however,



FIGS. 12-26 — (*b*, bract; *p*, petal; *s*, sepal; *st*, stamen). L.s. young flowers of *Nuphar lutea*, *N.* × *intermedia* and *N. pumila* in approximately comparable stages of development. The inception of the bract can be seen in Figs. 13, 18 and 23. From Figs. 14, 15, 19, 20, 24 and 25 it can be seen that the floral meristem of *N. pumila* grows away from the bract primordium sooner than that of *N. lutea*, *N.* × *intermedia* being intermediate in this respect. At the stamen-forming stage of floral development, the bract in *N. lutea* is still a distinct organ, whereas in *N.* × *intermedia* and *N. pumila*. it is only a parenchymatous swelling (Figs. 16, 21, 26). All. × 27.

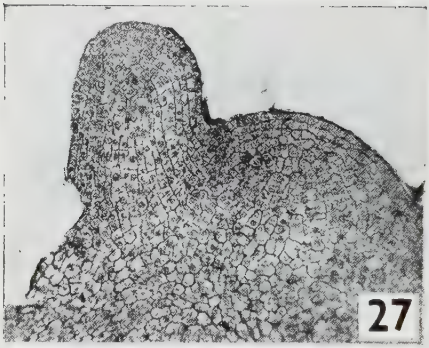
show that in these species the prevascular strand terminates some distance beneath the bract primordium, and is never present within the tissues of the bract (Figs. 38-43). As a result of growth, the parenchymatous projection which in older flowers represents the bract is considerably removed from the underlying vascular strand (Figs. 40, 43). There appears to be a correlation, therefore, between the amount of development that the bract undergoes and its degree of vascularization throughout its ontogeny.

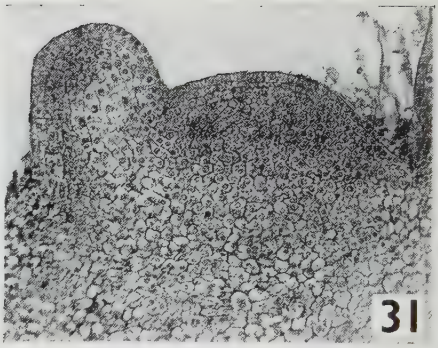
Discussion

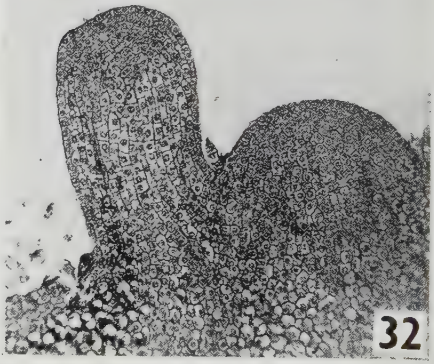
These studies of floral ontogenesis afford an example of the early manifestation in the apical meristem of somewhat different genetic effects in related species. It may be noted that the morphological differences associated with bract development in these two species of *Nuphar* and the hybrid between them would not have been correctly detected by even the most careful examination of mature, or nearly mature, stages. Differences in the development of the bract are most evident shortly after its inception. Bract inception in the three species (and in *N. advena* also) is similar, and takes place in flowers of comparable age, as measured in plastochrones, i.e. P_3 to P_5. The inception of sepals in *N. pumila*, *N.* × *intermedia* and *N. lutea* also occurs in flowers of comparable age as far as can be established from the more limited amount of material of the two former species examined. In these species, as in *N. lutea* (Cutter, 1957), therefore, there must be a considerable lapse of time between the inception of the bract primordium and that of the sepals. Thereafter the inception of the five sepals follows relatively rapidly. This is well seen in a specimen of *N. pumila* in which P_4, P_6 and P_8 were flower primordia. On P_4 an incipient bract primordium could just be distinguished; P_6 had a clearly discernible bract primordium, but no distinct sepals; and P_8 had four sepal primordia, the bract being now indistinguishable from the general abaxial ridge. In another specimen, P_7 had two sepal primordia only, while P_9 had all five sepal and several petal primordia. Petal formation therefore follows sepal formation with little delay. In comparing the relative sizes of the bract in different species, it must be remembered that the flower of *N. pumila* is smaller in all its parts than that of *N. lutea*.

In all the species of *Nuphar* examined, that part of the peduncle which subsequently elongates so greatly and carries the flower above the surface of the water is formed between the primordium of the bract (or bracts) and those of the sepals. From Figs. 14, 15, 19, 20, 24 and 25 it can be seen that in *N. pumila*, and to a lesser extent in *N.* × *intermedia*, elongation of this part of the peduncle occurs at a considerably earlier stage than in *N. lutea*; as a consequence, the bract primordium remains in proximity to the floral meristem for only a relatively short time. This may account, in part, for its comparatively slight development. It is known that in the vegetative stages of many plants the longer a leaf primordium remains near the apex, the slower its rate of development and the greater the morphological complexity it eventually attains.

In both *N. lutea* and *N. pumila*, however, the presence of bracts in adaxial, lateral or lateral-adaxial positions at the base of the mature peduncle has been observed. In *N. pumila* these were of variable size and form, but were usually larger than the abaxial bract, which indeed was generally invisible at this stage. In *N. lutea* the two bracts were more comparable in size; sometimes the abaxial one was the larger, and sometimes the other. Additional bracts in *N. lutea* were previously interpreted by supposing that two primordia were occasionally formed below the region of the petiole which subsequently elongates, instead of only one, and this was actually observed in young flowers (Cutter, 1957); and examples of abnormal flowers of several species of *Nymphaea*, in which one or more of the sepals had developed as bract-like organs at the base of the peduncle, were cited (Eichler, 1878; Glück, 1924; Planchon, 1851). It was further suggested that the differences in development between the bract and the sepals subsequently formed on the same floral meristem might be







FIGS. 27-34.

explicable in terms of the physiological and nutritional changes that had taken place in the meristem during the time between bract and sepal inception. Surgical experiments on the young developing flowers of *Primula bulleyana* have shown that the floral apex does undergo a series of changes, and that a new apex formed from one that has attained a certain stage will form only the organs appropriate to that and subsequent stages (Cusick, 1956). In flowers of *Nuphar*, bracts are formed in the pre-sepal stage. If the foregoing argument is true, it might be expected that bracts formed just prior to sepal formation might develop further than those formed at an earlier stage. Yet their primordia would have been associated with the floral meristem for an even shorter time than that of the first, abaxial, bract; consequently it appears that this factor must be relatively unimportant. It seems likely that the adaxial and lateral bracts of *N. pumila* were formed appreciably later than the abaxial bracts, just before the elongation of the peduncle; and the fact that they were often adnate to the peduncle over part of their length may be considered to support this view. Unfortunately, since these supernumerary bracts in *N. pumila* were observed on the peduncles of expanded flowers, nothing is known of the arrangement of the sepals in these specimens.

From Figs. 35-43 it is clear that whereas the bracts of *N. lutea* possess a prevascular strand which traverses the whole of the bract, approximately from the time of its inception, those of *N. pumila* and *N.* × *intermedia* have no such strand and, indeed, become progressively further removed from the underlying vascular tissue. The absence of vascular tissue within the bract is correlated with the poor growth of the latter; but it is less clear which is the cause and which the effect. It may be noted that the later stages of bract development in *N. pumila* and *N.* × *intermedia*, both of which lack vascular tissue in the bract, are much more similar than the intermediate stages of ontogenesis. A somewhat parallel situation with regard to vascularization exists in the microphylls of members of the Psilophytales (Bower, 1935).

On the whole, it seems likely that the genetic factors controlling bract development in these two species of *Nuphar* and their hybrid exert their principal effect just subsequent to the inception of the bract primordium, possibly in part by affecting differential growth in the young flower. At this stage the bract primordium of *N.* × *intermedia* appears intermediate between those of its parents. For whatever reason, the bract primordia of *N.* × *intermedia* and *N. pumila* evidently have little capacity for further growth, and no prevascular tissue is formed within them; parenchymatization then ensues and the bract of *N.* × *intermedia* comes to resemble that of *N. pumila* more than that of its other parent, *N. lutea*. Occasionally, however, further growth of the bract does take place, and *N. pumila* may possess, at maturity, a bract comparable in size with that of *N. lutea* (Fig. 2). The processes under genetic control must therefore be sufficiently flexible to allow for these fluctuations. The genetical aspect of this investigation will not be further discussed at this stage.

In the light of the additional information presented in this paper, it may be worth considering again the morphological nature of the bract in *Nuphar*. Previously it was pointed out that, since it could be shown that the bract was the first primordium of the floral meristem and was not a product of the rhizome apex, the flowers of *Nuphar*

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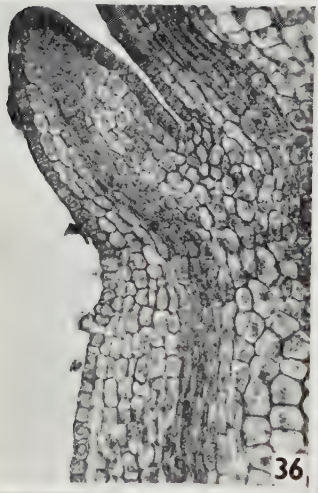
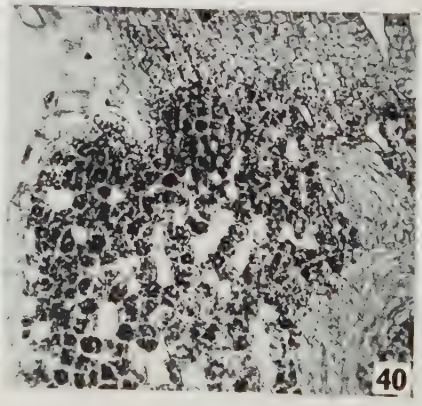
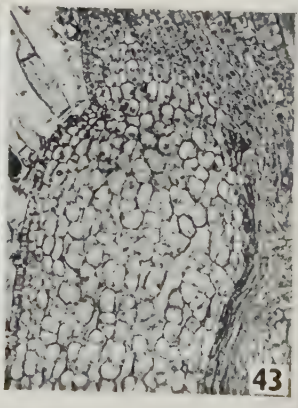
FIGS. 27-34 — Figs. 27-30. L.s. young flowers of *Nuphar* × *intermedia*, at the stages, respectively, of P_3, P_4, P_5 and P_7, showing the inception and development of the bract primordium (left). On P_3 (Fig. 27) the inception of the bract has not yet taken place. Figs. 31-34. L.s. young flowers of *N. pumila*, at the stages, respectively, of P_3, P_4, P_5 and P_7, showing the inception and development of the bract primordium (left). On P_3, from a specimen from Aviemore, the inception of the bract is just taking place (Fig. 31). Compare Figs. 27-30. All. × 100.







FIGS. 35-43.

must be considered to occur in leaf sites and not as axillary organs, as previous workers had interpreted them. It might be argued, however, that the bract was a subtending leaf initially adnate to the flower, i.e. that the floral meristem before the stage of P_3 was a composite structure consisting of the primordia of the flower and of its subtending leaf, resembling the floret primordia of some cereals (Purvis & Gregory, 1937). The fact that, unlike those of *Nymphaea*, the vegetative buds of *Nuphar* are axillary might be considered to support this view. However, the occasional occurrence at the base of a peduncle of two bracts, which in *N. lutea*, at least, are morphologically similar, seems to render this interpretation invalid.

Raciborski (1894) considered that the small bract of *N. advena* could be regarded as such only on the grounds of its analogy with the bract of *N. lutea*. In the present paper it has been shown that the bracts of *N. pumila* and *N.* × *intermedia*, although originating in the same way, usually undergo so little development that they are not detectable in the mature state. Previously it was considered that the term ' bract ' could be retained for the organ at the base of the peduncle in *N. lutea* and *N. advena* (Cutter, 1957), this term being taken in its broad sense, as signifying a leaf-like organ which differs from the foliage leaves and is associated with flowers (Rickett, 1954). The fact that in some species of *Nuphar* the bract develops to a greater extent than in others does not really affect the morphological nature of that organ. However, if the term ' bract ' is to be retained in this connection, it must be clearly defined. The ' bract ' in *Nuphar* is the first-formed lateral organ of the floral meristem, which because it is formed early, below the elongating region of the peduncle, later comes to be situated at the base of the peduncle; in different species of *Nuphar* it undergoes a variable degree of development. It is homologous with the anterior sepal of the flowers of *Nymphaea*, and indeed probably has greater affinities with sepals than with any other organs. There is possibly a case for the introduction of a new term, rather than the retention of the term ' bract ', but the multiplication of technical terms to meet specific requirements is probably best avoided.

Finally, the question of the phylogenetic importance, if any, of these findings may be considered. Raciborski (1894) derived a phylogenetic series from the ' rudimentary ' bract of *Nuphar lutea* and *N. affine*, through the still more rudimentary swelling at the base of the peduncle in *N. advena*, to the bractless flowers of *Nymphaea*. This view has already been discussed elsewhere (Cutter, 1957). It is clear that a series —

←—

FIGS. 35-43 — L.s. young flowers of *Nuphar* spp. at various stages of development, showing the relationship of the bract primordium with the prevascular tissue. Fig. 35. *N. lutea*. L.s. flower at P_7, showing the prevascular strand within the bract primordium. Compare Figs. 38, 41. × 167. Fig. 36. *N. lutea*. L.s. bract from a flower at the petal-forming stage, showing prevascular tissue within the bract. Compare Figs. 39, 42. × 167. Fig. 37. *N. lutea*. L.s. bract from a flower at the ovary-forming stage, showing the prevascular strand within the bract. Compare Figs. 40, 43. × 100. Fig. 38. *N.* × *intermedia*. L.s. flower at P_7, showing prevascular tissue beneath, but not within, the bract primordium. Compare Figs. 35, 41. This flower (from Kew) was fixed in winter, and the abundant starch grains are stained black. × 167. Fig. 39. *N.* × *intermedia*. L.s. bract from a flower at the petal-forming stage, showing the prevascular tissue beneath the bract. Compare Figs. 36, 42. × 167. Fig. 40. *N.* × *intermedia*. L.s. bract from a flower at the ovary-forming stage, showing the prevascular tissue some distance away from the parenchymatous projection which represents the bract. Starch grains are stained black. Compare Figs. 37, 43. × 100. Fig. 41. *N. pumila*. L.s. bract from a flower at P_7, showing the prevascular strand beneath, but not within, the bract primordium. Compare Figs. 35, 38. × 167. Fig. 42. *N. pumila*. L.s. bract from a flower at the petal-forming stage, showing prevascular tissue beneath the bract. Compare Figs. 36, 39. × 167. Fig. 43. *N. pumila* (Aviemore). L.s. bract from a flower at the ovary-forming stage, showing prevascular tissue some distance away from the parenchymatous projection which represents the bract. Compare Figs. 37, 40. × 100.

although a rather different one — based on early floral ontogeny can indeed be considered to exist, and since, in de Beer's (1951) view, phylogeny is due to modified ontogeny, this may be of phylogenetic importance. The series can be based on the duration of the proximity of the first lateral member to the floral meristem which gives rise to it, and the degree of development of the former, and within the species investigated would run: *Nuphar pumila*, *N.* × *intermedia*, *N. advena*, *N. lutea*, *Nymphaea* spp.— thus placing *Nymphaea* at the opposite end from its position in Raciborski's series. It is possible that the series could be extended through the various species of *Nymphaea*; for example, Conard (1905) noted that the anterior and lateral sepals of the flowers of *N. lotus* were formed considerably earlier than the posterior sepal, and that the anterior sepal was inserted much lower on the peduncle. Even if this series is of phylogenetic significance, however, it is clearly only one criterion, and may well be only a minor one. Nevertheless, it should be emphasized that the existence of such a series — and the apparent mode of action of certain genetic factors — could not have been detected without investigating the earliest stages in floral ontogeny.

Summary

In a comparative developmental study of the flowers of *Nuphar lutea*, *N. pumila* and the naturally occurring hybrid between them, *N.* × *intermedia*, it was established that the bract previously known to be present in *N. lutea* occurred in the other species also. At the time of its inception as the first lateral member of the floral meristem, the bract primordium is comparable in all three species, but in *N.* × *intermedia* and especially *N. pumila* it undergoes little further growth, and develops as a hair-covered parenchymatous outgrowth which is not easily detectable externally. Indeed, bracts were observed on less than 20 per cent of the mature peduncles of *N. pumila* and *N.* × *intermedia*, although initially present on all flowers. Vascular tissue was present within the bracts of *N. lutea* at all stages of development, but not in those of *N. pumila* and *N.* × *intermedia*; there is thus a correlation between the amount of development of the bract and its degree of vascularization. The occurrence of additional adaxial or lateral outgrowths at the base of the peduncle in *N. pumila* is described.

I have pleasure in thanking Mr. G. Barker for taking the photographs.

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THE DEVELOPMENT OF THE EMBRYO IN *PAEONIA* — A REINVESTIGATION

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In angiosperms the first division of the zygote is always followed by wall formation. This is in marked contrast with the condition in gymnosperms where the first few divisions are almost always free nuclear, the chief exceptions being *Sequoia* and *Gnetum*. Rutgers (1923) reported a free nuclear embryo in an angiosperm *Moringa oleifera*, but Puri (1941) showed that the apparently coenocytic mass is merely a micropylar accumulation of endosperm nuclei.

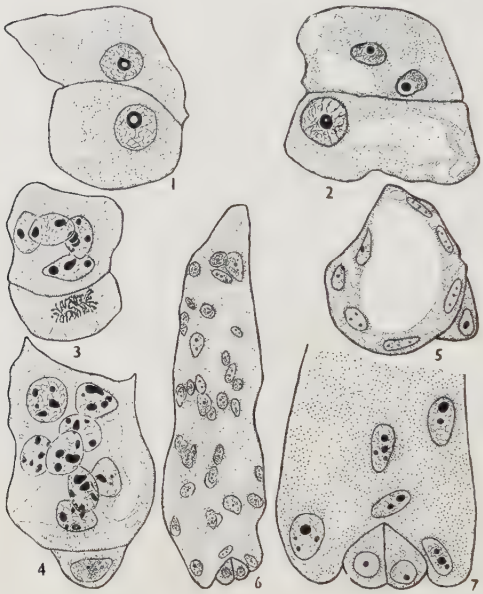
Recently, in a paper entitled "On some peculiar features in the embryogeny of *Paeonia* L.", Yakovlev & Yoffe (1957) reported a type of embryo development which they claim to be remarkably similar to that of certain gymnosperms like *Ginkgo*. According to them, the first division of the zygote is always unaccompanied by wall formation. The two free nuclei thus formed divide repeatedly without wall formation, resulting in a number of free nuclei which become evenly distributed along the periphery of the vesicular coenocyte while the centre is occupied by a large vacuole. Wall formation is said to take place only at a later stage. The resultant mass of cells does not differentiate directly into an embryo. Instead, certain peripheral cells form meristematic centres which protrude and give rise to embryonal primordia. While there may be several such primordia, normally only one attains maturity.

The novelty of this account prompted me to undertake a study of the embryo development in several special of *Paeonia* (*P. albiflora*, *P. actiflora*, *P. delavayi*, *P. suffruiticosa* and an undetermined species). Surprisingly, my findings do not support the interpretations of Yakovlev and Yoffe. In all the species studied by me, the first division of the zygote is definitely followed by wall formation, resulting in a two-celled proembryo (Figs. 1, 8, 9). Free nuclear divisions occur only in the basal cell which forms a greatly hypertrophied suspensor (Figs. 2-4, 6). The nuclei in this cell occupy a peripheral position leaving a central vacuole (Fig. 5). The length and shape of the suspensor cell vary in different species.

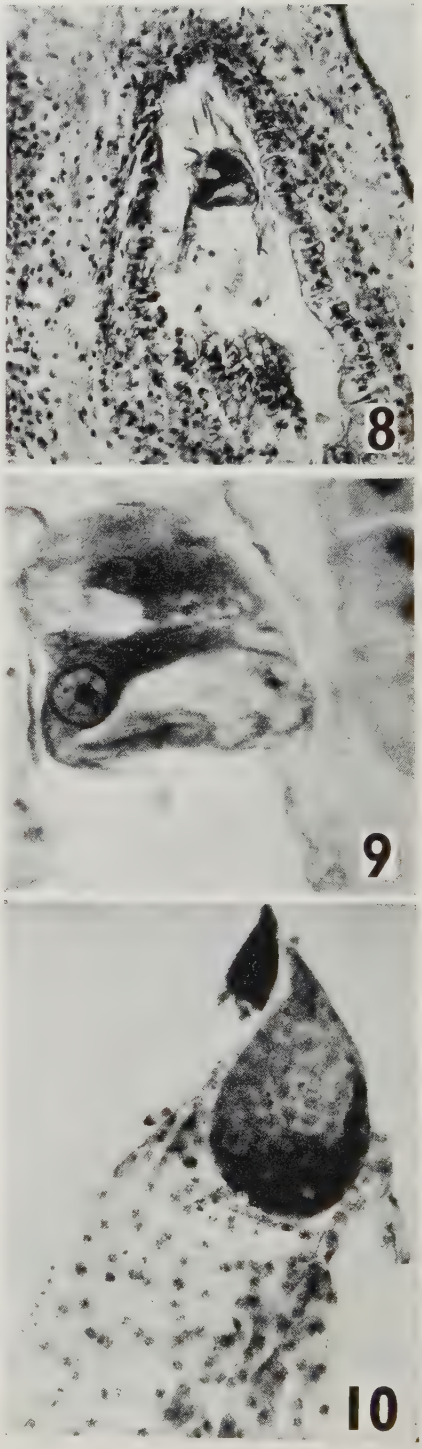
The apical cell usually divides after the suspensor cell has attained a fairly large size. The first division is followed by a vertical wall (Fig. 7), but this is not always so. The spindle in Fig. 3 is oriented in such a way so as to result in a transverse wall. Yakovlev and Yoffe seem to have overlooked the apical cell and mistaken the coenocytic suspensor for the embryo proper. This is because of the massive growth of the suspensor which runs in several sections and is thus a far

more conspicuous part of the embryo. The apical cell which is much smaller can be seen only in a median section.

After producing several hundred nuclei the multinucleate suspensor cell too enters a cellular phase. Walls are laid down from the periphery inwards and eventually the whole of the coenocyte becomes cellular. Meanwhile the apical cell also



FIGS. 1-7 — Fig. 1. *Paeonia* sp., 2-celled proembryo. × 250. Fig. 2. *P. actiflora*, the basal cell shows two free nuclei. × 250. Fig. 3. *P. delavayi*, there is a four-nucleate basal cell and undivided apical cell. × 250. Fig. 4. *P. delavayi*, 8-nucleate basal cell and undivided apical cell. × 250. Fig. 5. *P. albiflora*, apical cell lying on one side, the nuclei in the coenocyte are arranged peripherally. × 250. Fig. 6. *Paeonia* sp., large suspensor haustorium; the apical cell has divided vertically into two cells. × 111. Fig. 7. Magnified view of portion of Fig. 6. × 250.



FIGS. 8-10.

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FIGS. 8-10 — Fig. 8. *P. actiflora*, upper part of fertilized embryo sac showing a proembryo. × 75. Fig. 9. Magnified view of the two-celled proembryo in Fig. 8; note the two nuclei in the basal cell. × 440. Fig. 10. *P. albiflora*, whole mount showing the presence of twin embryos formed from two different embryo sacs. × 310.

embarks upon a series of cell divisions so that in later stages it is not easy to demarcate the derivatives of the basal and the apical cell.

Yakovlev and Yoffe figured certain peripheral meristems as giving rise to embryonal primordia. This too is not confirmed in my material although I did see a few ovules showing false polyembryony (Fig. 10) caused by the growth of two embryo sacs in the same ovule.

Briefly then, contrary to Yakovlev and Yoffe, the embryo of *Paeonia* like any other angiosperm is cellular and *not* free nuclear and the coenocytic structure misinterpreted by them as the free nuclear embryo is in reality the massive suspensor haustorium.

I am greatly indebted to Professor P. Maheshwari under whose valuable guidance this research was carried out and to Dr R. C. Sachar for help and interest. The observations recorded in the paper are based on materials of *Paeonia* obtained by Professor P. Maheshwari from various countries through the courtesy of Professors Th. Eckhart, O. Hagerup, E. Söderberg, P. Crété, H. Kihara, M. Kumazawa, M. Ernst-Schwarzenbach, N. Higinbotham, T. M. Harris, and W. A. Van Heel; and Drs Y. Nozu, B. M. Johri, Nirmal Kapil, A. N. Rao and B. Saha. To all these I am greatly indebted for the pains taken by them. Materials sent by some other botanists are still under investigation.

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DEVELOPMENT OF THE SHOOT OF *ORYZA SATIVA* L.— II. LEAF HISTOGENESIS

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Historical Review: Gross Morphology and Histologic Development of the Grass Leaf

The initiation and development of the leaf primordium have been studied in considerable detail by many investigators. Before the pertinent literature is reviewed, the term *leaf primordium* should be defined. With reference to grasses the writer will use it in this sense: the leaf primordium is a lateral appendage of the axis of the shoot, which is initiated from the tunica and/or corpus of the apical meristem and undergoes transition from primordial to young leaf stages of development with the initiation of the ligule (with or without auricles). Evans & Grover (1940) adopted the idea that in grasses a leaf primordium becomes a young leaf when it exceeds a length of one millimeter and has circumscribed at least one-half of the axis. According to my opinion, the leaf primordium, when it becomes a young leaf,

completely encloses the axis with overlapping margins and is several centimeters high.

Another term which is used in connection with studies of the origin and development of leaf primordia is *plastochron*, a term coined by Askenasy (1880), and now commonly used to designate the time intervening between the initiation of two successive leaf primordia (e.g. Sharman, 1942a; Abbe & Phinney, 1951; Erickson, 1957; Michelini, 1958). With regard to the Gramineae Evans & Grover (1940) use plastochron to indicate "the number of days required for the initiation and organization of a phytomer of a grass shoot up to the time a discernible leaf primordium has developed on it, not to the time of leaf emergence or expansion."

The gross morphology of the leaf primordium in grasses has been treated in detail (Sontag, 1887; Deinega, 1898; Goebel, 1905; Noguchi, 1929; Bonnett, 1935, 1937, 1940; Troll, 1939; Evans & Grover, 1940; Sharman, 1942a, 1942b, 1945, 1947; Kiesselbach, 1949; Abbe & Phinney, 1951; Hubbard & Leng, 1955). This work will not be reviewed here except as it pertains to morphologic development of leaf primordia in grasses.

Variations in the number and position of primordial leaf protuberances (cf. definition on p. 277) occur at different stages of development in individual species and between different species of grasses (Evans & Grover, 1940; Sharman, 1942a). Vegetative plants of some grasses may possess relatively few primordial leaf protuberances, whereas in the same plants, at transition to flowering stage of development, a much larger number is present. Leaf primordia in grasses are initiated from the apical meristem according to a distichous arrangement. The correct number of primordial leaf protuberances can thus be assessed by examining living or sectioned shoots in the plane of insertion of the leaves. In different species of grasses the maximum number of primordial leaf protuberances observed at any one time varies; e.g. seventeen in perennial ryegrass, five or six in orchard grass, and one in rice.

In the Gramineae the initiation of a leaf primordium is marked by periclinal divisions in the outermost tunica layer; e.g. *Dactylis glomerata* (Bugnon, 1921), *Triticum vulgare* (Rösler, 1928; Pankow & Guttenburg, 1959), *Avena sativa* (Kliem, 1937; Hamilton, 1948), *Sinocalamus beecheyana* (Hsü, 1944), and various other grasses (Schüepp, 1926; Thielke, 1951; Roth, 1957).

In grasses the regions of the apical meristem which participate in the formation of leaf primordia are variable: the tunica alone in grasses having a biseriate tunica, the uniseriate tunica and corpus in *Sorghum bicolor* and *Oplismenus imbicillis* var. *variegatus* (Thielke, 1951); and the uniseriate tunica alone in *Dactylis glomerata* and *Zea mays* (Thielke, 1948b).

Two distinctive trends in the relative cellular contribution of the tunica of the apical meristem to the leaf primordium are described in the literature: in many monocotyledons the outermost layer of the tunica divides anticlinally and periclinally in the locus of a leaf primordium and thus contributes to the ground meristem as well as to the protoderm of the primordium (Herrig, 1915; Rösler, 1928; Pottier, 1934; Foster, 1936b, 1949; Kliem, 1937; Rüdiger, 1939; Sharman, 1942b, 1945; Hsü, 1944; Hamilton, 1948; Thielke, 1948a, 1951); in others, the single-layered tunica divides anticlinally and thus contributes only to the protoderm (Herrig, 1915; Foster, 1936b, 1949; Rüdiger, 1939; Schalscha-Ehrenfeld, 1940; Sass, 1944; Stant, 1952).

The primary meristematic tissues of the leaf primordium in grasses usually become demarcated during the first plastochron. All available data suggest that the median procambial strand first appears at a point some distance below the region of insertion of the leaf primordium and develops acropetally into the primordium during the first plastochron (Deinega, 1898; Sharman, 1941, 1942b; Hsü, 1944). Hsü indicates that in *Sinocalamus beecheyana* the median procambial strand of the leaf primordium also differentiates basipetally into the stem from the region of its first appearance, the region of insertion of the leaf. Lateral procambial strands, also originating in the leaf base, develop acropetally into the leaf primordium and basipetally into the stem (Sharman,

1942b). The protoderm of the leaf primordium is derived from the tunica of the apical meristem. According to Sharman (1942b), continued anticlinal divisions at the free edge of the leaf primordium add tissues to the tip and margins of the collar-shaped primordium. Periclinal divisions have been reported in the protoderm at the apex of the primordium (Hsü, 1944; Sharman, 1945).

The ground meristem is at first homogeneous in cytohistology and is composed of more or less isodiametric cells. Later some cells of the ground meristem differentiate into procambial strands (the same as those mentioned above); others become organized into the mariginal meristems at the flanks of the primordium; and those in the future midrib region become organized into a rib meristem. Concomitantly, cells of the protoderm and ground meristem on the abaxial side of the leaf primordium divide anticlinally more rapidly than those on the adaxial side, a method of growth resulting in the assumption of dorsiventral symmetry by the primordial leaf (Deinega, 1898; Sharman, 1942b, 1945; Hamilton, 1948).

In connection with apical growth of leaf primordia in grasses Sontag (1887) is of the opinion that apical growth is initiated in the primordium before it is 0·3 mm high; that apical growth is relatively unimportant during the time when intercalary growth is active. According to him, apical growth is completed when the length of the primordial leaf is 0·36 mm in *Lolium italicum*, 0·5 mm in *Phragmites communis*, and 0·6 mm in *Luzula maxima*. Sharman (1941) has illustrated the relative contribution of protoderm and ground meristem to the apical growth of the leaf primordium in *Agropyron repens*.

According to many investigators, the ligule of the grass leaf is of protodermal origin (Goebel, 1884; Bugnon, 1924; Pottier, 1934; Philipson, 1935; Neumann, 1937; Rüdiger, 1939; Sharman, 1941, 1942b, 1945; Hamilton, 1948; Thielke, 1948b). For *Zea mays* and other grasses Sharman (1941) indicates that the ligule is initiated "by rapid periclinal divisions in a few young, rather more densely staining epidermal cells at the union of the future lamina and sheath," and that in maize (based on work of Neumann, 1937) "'three horizontal rows of cells lying one over the other'" are specifically the cells involved in this process These findings are supported by the work of Bugnon (1924), Ponzo (1931), Philipson (1935), and Neumann (1937). Very little information is available on later stages of ligule development, ligule vascularization, and initiation of auricles, and apparently none on the development of auricles.

The gross morphological development of the grass leaf, after the primordial leaf stage, has not been thoroughly treated in the literature. Instead, emphasis has been placed upon the morphology of mature leaves (Trécul, 1853; Duval-Jouve, 1875; Pée-Laby, 1898; Deinega, 1898; Goebel, 1905; Bugnon, 1921; Arber, 1925, 1934; Hector, 1936; Artschwager, 1940, 1948; Hitchcock, 1950). There is also very little information on growth pattern concepts of the rib, plate, and intercalary meristems in relation to the development of grass leaves.

Marginal growth of angiosperm leaves has been the subject of intensive and extensive investigations (Foster, 1936b, 1949; Troll, 1939; Gifford, 1951). Most of this work applies to dicotyledons and, to my knowledge, the researches of Pottier (1934), Renner (1936b), Renner & Voss (1942), Sharman (1942b, 1945), Thielke (1948a), Mericle (1950), and Pray (1957) are the primary ones pertaining to monocotyledons.

I have not encountered any detailed histological treatment on marginal meristems of leaf primordia except that of Thielke (1948a) on marginal growth in variegated leaves of various monocotyledons and dicotyledons. Sontag (1887), reporting on the grasses, *Lolium* and *Phragmites*, mentions that marginal growth proceeds basipetally from the tip of the primordium. Esau (1953) points out that upward growth of the margins of the leaf is marginal growth; that the processes of apical and marginal growth are not as distinct in the monocotyledon leaf as in dicotyledon leaves; and that these two types of growth are of comparatively short duration, whereas intercalary growth, at the base of the leaf,

continues after the former have ceased, causing further elongation of the leaf.

Usually a series of marginal initials exclusively gives rise to the protoderm of the leaf. Lund's type of marginal meristem which he characterizes as "marginal initials of the 'third degree', from which new cells are formed by anticlinal, non-convergent walls, resulting in a single, continuous layer of cells or 'dermatogen'" (*see* Foster, 1936a) is characteristic of the marginal meristem of the lamina of *Zea mays* (Mericle, 1950) and the rice plant (present work). Notable exceptions to the above are recorded by Foster (1937), Renner (1936a, 1936b), Renner & Voss (1942), Sharman (1942a, 1945), Pottier (1944), and Thielke (1948a); in these instances, a marginal initial may divide periclinally and contribute to the internal tissue of the young leaf as well as to the protoderm.

Various schemes have been presented to illustrate derivatives of marginal meristems (Foster, 1936b; Mericle, 1950; Gifford, 1951). Gifford (1951) distinguishes three types of activity of submarginal initials. The schematic diagram of Mericle (1950) for the leaf of *Zea mays* represents a modification of Gifford's third type. In this type submarginal initials divide to form adaxial and abaxial layers or a single internal layer, which in turn produces the adaxial and abaxial layers; the adaxial layer produces the palisade parenchyma, the vascular bundles, and part of the spongy parenchyma. In *Zea* this layer produces a middle layer, and it is from the latter that procambial strands are formed.

The only comprehensive treatment of vascularization of a grass leaf is that given for *Zea mays* by Sharman (1942b). The salient details have been summarized by Esau (1953).

Form and Structure of the Rice Leaf[1]

The study of the gross morphology of the rice leaf included investigation of external morphology and anatomy of the leaf and quantitative analysis of leaf elongation during successive plastochrons.

1. For information on materials and methods, see first paper of this series (Kaufman, 1959).

Some aspects of the gross morphology and histology of the mature rice leaf have been treated in detail by several investigators (Duval-Jouve, 1875; Pée-Laby, 1898; Goebel, 1905; deHaan, 1911; Arber, 1934; Tullis, 1935; Hector, 1936; Juliano & Aldama, 1937; Duong-Huu-Thoi, 1941). The observations of these workers are summarized below along with the present work.

The concept of leaf or phyllome, in connection with the rice plant, would include the scutellum (the cotyledon), coleoptile, and foliage leaf. The scutellum and coleoptile each consist of only one lamina-like part, and the third leaf on the plant (first after the coleoptile) has a diminutive lamina but no ligule and auricles or sheath. The foliage leaf (fourth and successive leaves) is composed of lamina and sheath with a ligule and two auricles interposed at the transition region between these two parts.

Gross Morphology of the Sheath — Above its region of insertion the sheath encircles all or part of the internode. At the base of the plant tightly enwrapped leaf sheaths enclose the earliest formed internodes. Growth of the sheath is primarily basal and may continue after the upper regions of the sheath have ceased to elongate. The sheath is open longitudinally on one side from the ligule region to the base of the sheath and is hence a bifacial structure. There is no distinct differentiation of a midrib in the sheath as in the lamina. The surface of the sheath is finely ribbed or corrugated.

Anatomy of the Sheath — Cells of the adaxial and abaxial epidermal layers differ, in that those of the former are larger and of one type (long type). Several types of cells are characteristic of the abaxial epidermis; these include long cells with undulated walls, single or paired short cells, short and thick-walled unicellular trichomes, long two-celled trichomes, and pairs of guard cells flanked by lateral subsidiary cells and arranged in parallel longitudinal series.

The interior of the sheath is composed of a longitudinal system of cavities (lacunae) interrupted by transverse diaphragms, which consist of stellate parenchyma cells and commissural bundles.

These lacunae are distributed in the midrib and wings along the entire length of the sheath (Figs. 1, 4-8); and lacuna development in the horizontal direction proceeds from the central region to the margins of the wings (Fig. 4). A series of vascular bundles extends longitudinally through the ground parenchyma of the sheath. Large and small bundles alternate between the lacunae subjacent to the abaxial epidermis. They are connected to the transversely oriented commissural bundles. In the upper portion of the sheath the vascular bundles located in the wings proceed directly into the ligule and auricles. Bundle sheaths are continuous with ground parenchyma separating the vascular bundles and the air cavities. Masses of fibers occur in association with the vascular bundles and as isolated strands embedded in the ground parenchyma on the adaxial side of the sheath. The strand of fibers located on the abaxial side of each bundle is U-shaped (in cross section) near the base of the sheath and bar-shaped in the upper regions. Chloroplasts are present in mesophyll tissue throughout young sheaths, but after lacunae have formed, they disappear from the longitudinal plates of mesophyll cells separating the lacunae and from the adaxial mesophyll and are retained only in the abaxial mesophyll.

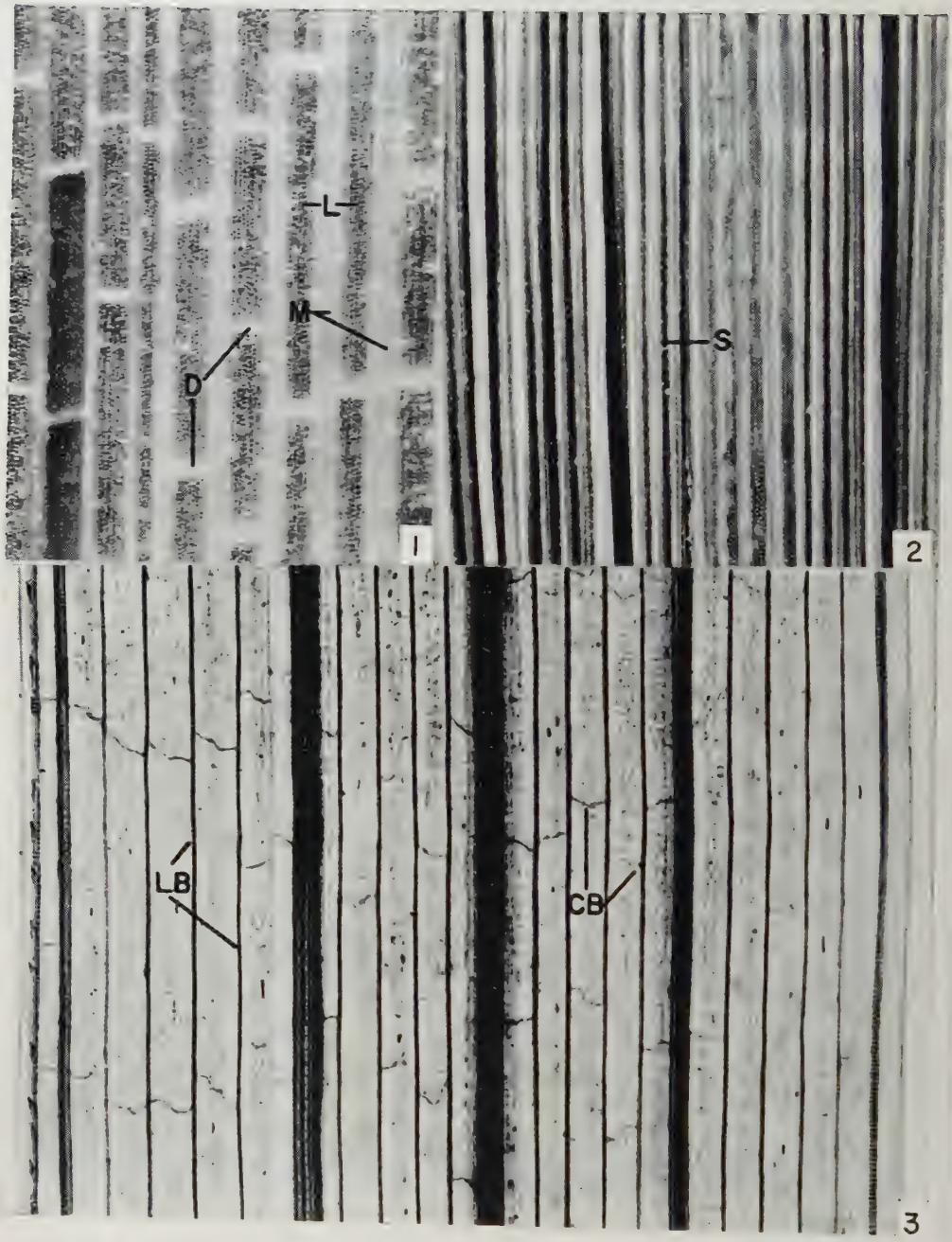
Iodine tests indicate that starch is absent in the sheath at very early and late stages of development, and when present, occurs only in bundle sheath cells and mesophyll tissue near the margins of the wings.

Anatomy of the Ligule Region — This part of the leaf consists of midrib only and is identified by the presence of a ligule and two auricles (Fig. 47). The lacunae of this region are in direct continuity with lacunae in the sheath below and the lamina above. Vascular bundles occur in both abaxial and adaxial parts of the midrib. Chloroplasts are distributed throughout the mesophyll parenchyma except in the dewlap region. In the dewlap, which is the short most basal part of the midrib of the lamina, located just above the ligule and auricles, the mesophyll cells appear to be devoid of chloroplasts.

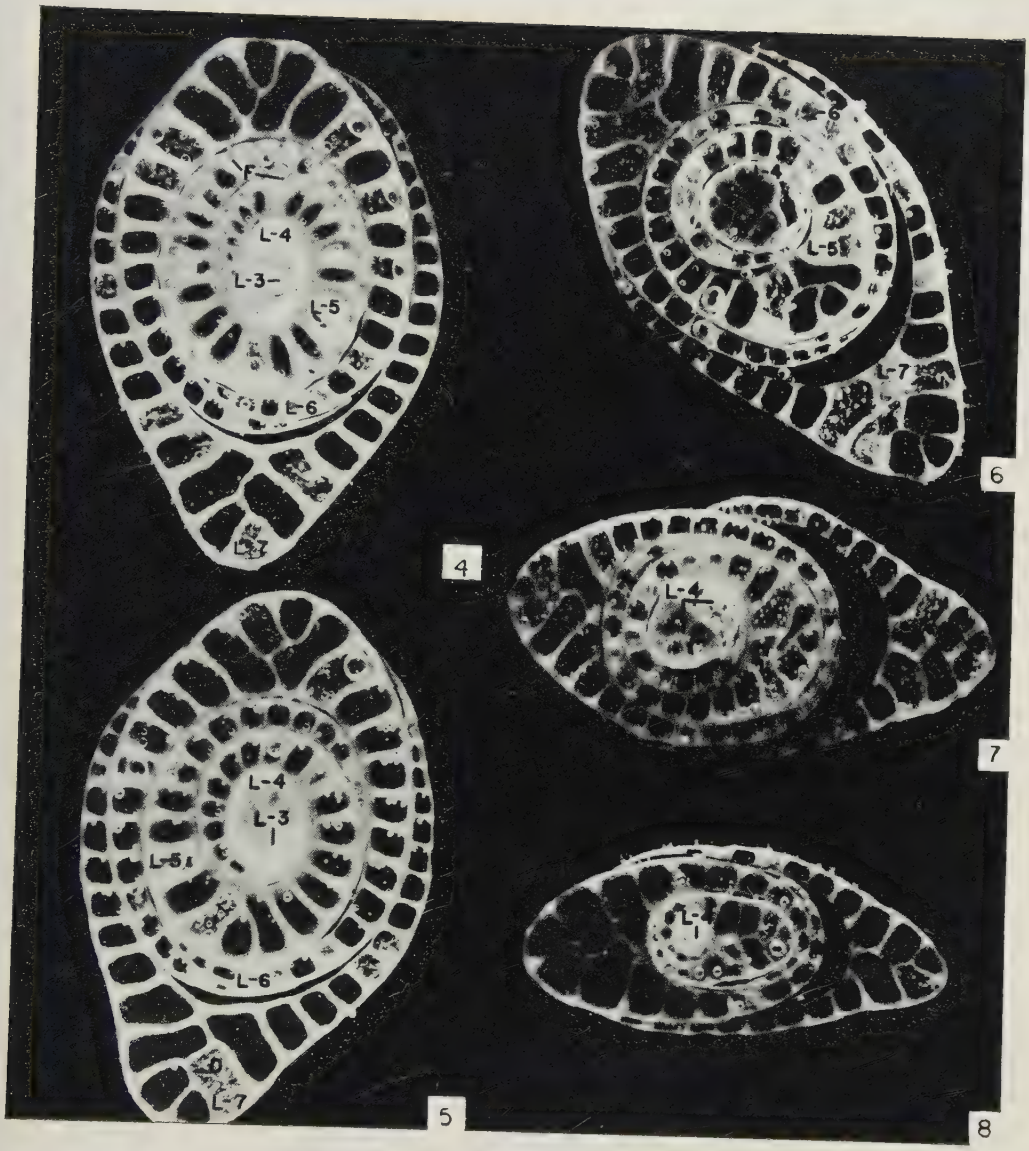
Gross Morphology of the Lamina — The lamina is composed of a midrib and two wings. The midrib is prominent in the basal region of the lamina and progressively diminishes in size toward the apical region. Just above this basal region extremely narrow wings are evident. These extend outward to a progressively greater extent up to the approximate middle part of the lamina. They then diminish in lateral extent from this central part up to the tip of the lamina. The topography of the adaxial surface of the lamina is depicted in Figs. 2, 3, 6-8.

Anatomy of the Lamina — The epidermis is histologically similar to the abaxial epidermis of the sheath, except that longitudinal bands of bulliform cells, alternating with vascular bundles in the mesophyll, are present in the adaxial epidermis of the wings. These cells are large and bulbous in form and hyaline. Overlying the epidermis is a papillate cuticle.

The internal structure of the lamina is considerably different from that of the sheath. Lacunae are limited to the midrib and are separated by large, thin-walled, colorless parenchyma cells. These lacunae extend from the ligule region upwards to about the middle of the lamina (Figs. 6, 7, leaf 4), the number varying from six to eight just above the ligule region, decreasing to four, then to two, and finally to none at successively higher levels in the lower half of the lamina. Lacuna development in young laminae proceeds basipetally from the center of the lamina midrib to the ligule region and extends into the sheath. Diaphragms in the lacunae are not oriented in perfectly horizontal planes but may be tilted at various angles. In the lamina midrib vascular bundles occur below both the abaxial and adaxial epidermis. The pattern of longitudinal and commissural bundles is shown in Fig. 3. Various cell complexes and structures associated with mature vascular bundles are rather conspicuous; namely, the bundle sheaths, bundle sheath extensions, protoxylem lacunae, the large metaxylem vessel elements, and sieve-tube elements of the metaphloem (Figs. 58, 59). Strands of fibers occur in association with the vascular



FIGS. 1-3 — (*CB*, commissural vascular bundles; *D*, diaphragms; *L*, lacunae; *LB*, minor vascular bundles of longitudinal system; *M*, mesophyll; *S*, stomata). Fig. 1. Adaxial surface of sheath. The longitudinal series of cells in the lacuna regions represent mesophyll parenchyma between these lacunae and the adaxial epidermis. The opaque longitudinally oriented strands of tissue are "ribs" of mesophyll parenchyma separating the lacunae. Two lacunae at far left have been cut open. × 12. Fig. 2. Adaxial surface of the lamina, showing longitudinal ridges associated with the vascular bundles. The series of light-colored dots are stomata. × 12. Fig. 3. Cleared segment of the lamina with view of adaxial surface. Unicellular trichomes are discernible at margins of the lamina, × 16.



FIGS. 4-8 — (*D*, diaphragm; *F*, strands of fibers; *L*-3 to *L*-7, leaves 3 to 7). All. × 12. T.s. at successively higher levels of the same shoot. Fig. 4. Section of shoot near apical meristem. Leaf 3, the innermost leaf, is represented by portion of the lamina; leaves 4 to 7 are sheaths of successively older leaves. Recently formed lacunae in enlargement stage are evident in leaf 5; fully expanded lacunae are present in leaves 6 and 7. Fig. 5. Section of shoot at level of ligule of leaf 4. Lacunae at this level of leaf 4 are more apparent than those at lower level of same leaf in Fig. 4; in leaf 5, lacunae are considerably wider than those of same leaf in Fig. 4. Fig. 6. Section just above apex of leaf 3. Leaf 4 is represented by portion of lamina; leaves 5, 6 and 7 by sheaths. Fig. 7. Section through lamina of leaf 4 at level approximately one-third distance from lamina base. At this level of leaf 4, lamina midrib has diminished in area; the wings are much wider and more folded; the number of midrib lacunae is smaller (compare with Fig. 6). Leaves 5, 6 and 7 are represented by sheaths. Fig. 8. Section just below ligule region of leaf 5 (third from outside). Leaf 4 is represented by section near upper half of lamina, where there is no midrib.

FIGS. 9, 10.

bundles on the abaxial and adaxial sides of the midrib and in the wings (Fig. 58). In the adaxial portion of the lamina wings, the strands of fibers are located just below the epidermis and adjacent to the ends of the bundle sheath extensions; in the abaxial portion, they are present between bundle sheaths and the abaxial epidermis (Figs 58, 59). Between the vascular bundles and strands of fibers in the lamina wings is the mesophyll tissue, in which the cells contain numerous chloroplasts (Figs. 9, 10, 59). The cells show infoldings as are characteristic of the type of cells called armed parenchyma cells.

Chloroplasts have a striking distribution pattern in the lamina; they occur in the adaxial and abaxial mesophyll parenchyma of the midrib and the armed parenchyma cells of the wings. They are conspicuously absent from the bundle sheath extensions, " ribs " of mesophyll parenchyma between lacunae, and the epidermal cells. At no time was starch observed in the lamina mesophyll.

Quantitative studies on lengths of leaf primordia, laminae, and sheaths of seedlings and older vegetative plants during successive plastochrons were carried out by means of weekly leaf measurements. Data were tabulated according to successive plastochrons (Table 1). The term plastochron is used in the sense of Abbe & Phinney (1951).

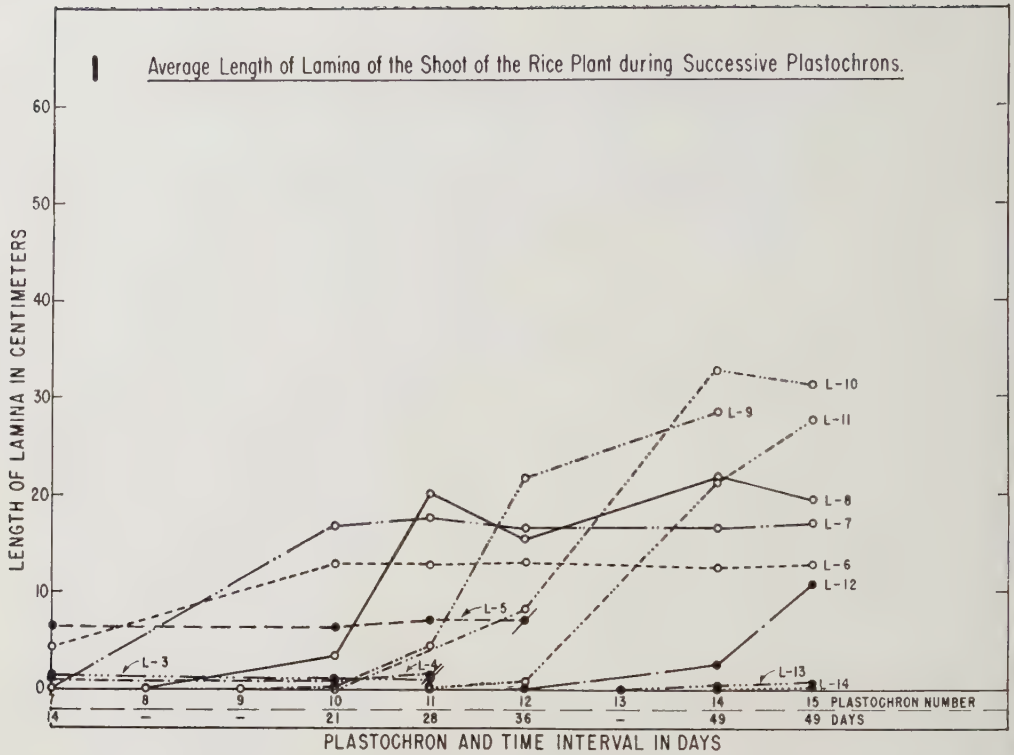
The following information is important for the study of the table: (1) the scutellum and coleoptile, considered as leaves 1 and 2 respectively, were not measured; (2) leaf measuremants were not obtained during plastochrons 1 to 6 due to inaccuracy of measurements (all mostly microscopic); (3) not all plastochrons between 7 and 15 are represented because data were obtained weekly, and during a seven-day interval, sometimes two plastochrons had elapsed; (4) lengths of the leaf primordium which forms a hood over the apical meristem were not recorded because accurate measurements of this structure could not be obtained; (5) in the tables, where there are data for the total leaf and no data for the lamina and sheath, or for lamina, sheath and total leaf, the given leaf, designated by symbol " *LP* ", was in the primordial phase of development; (6) where a given leaf or part of leaf had reached senescence, it is indicated in the table by " *S* "; (7) the presence of ligule and auricles is indicated by an " *L* " symbol in the space for sheath.

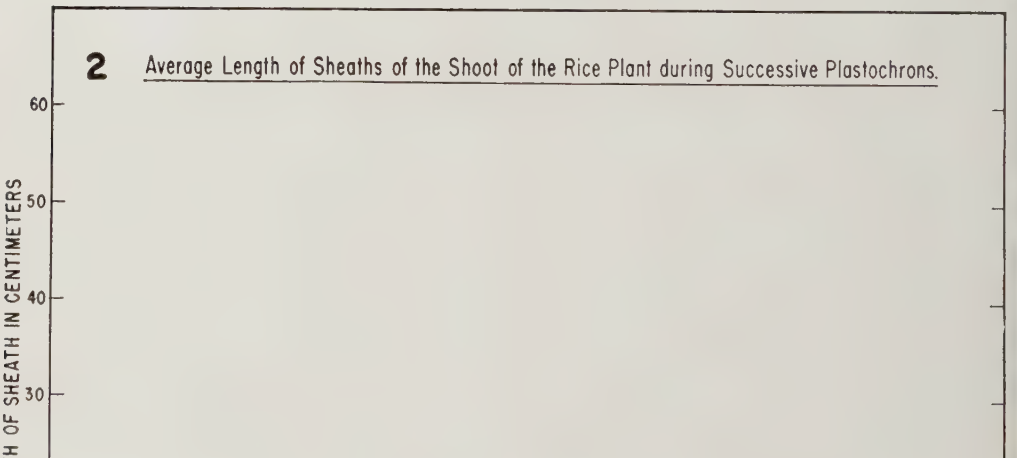
Table 1 gives the following information. The leaf sheath first becomes evident with the appearance of a ligule in the third leaf above the apical meristem. Leaf 3, the first so-called foliage leaf, has no sheath or ligule and auricles. It produces a small blade, varying in this material from 0·9 to 1·5 cm in length, reaches senescence during plastochron 11 or 12, and is sloughed off within the same plastochron. Leaf 4 produces a relatively long sheath (2·9 to 5·3 cm) as compared with its lamina (0·9 to 1·5 cm), reaches senescence during plastochron 11 or 12, and is sloughed by the fourteenth plastochron. In the succeeding older leaves which are represented in this table the lamina is progressively longer than the sheath.

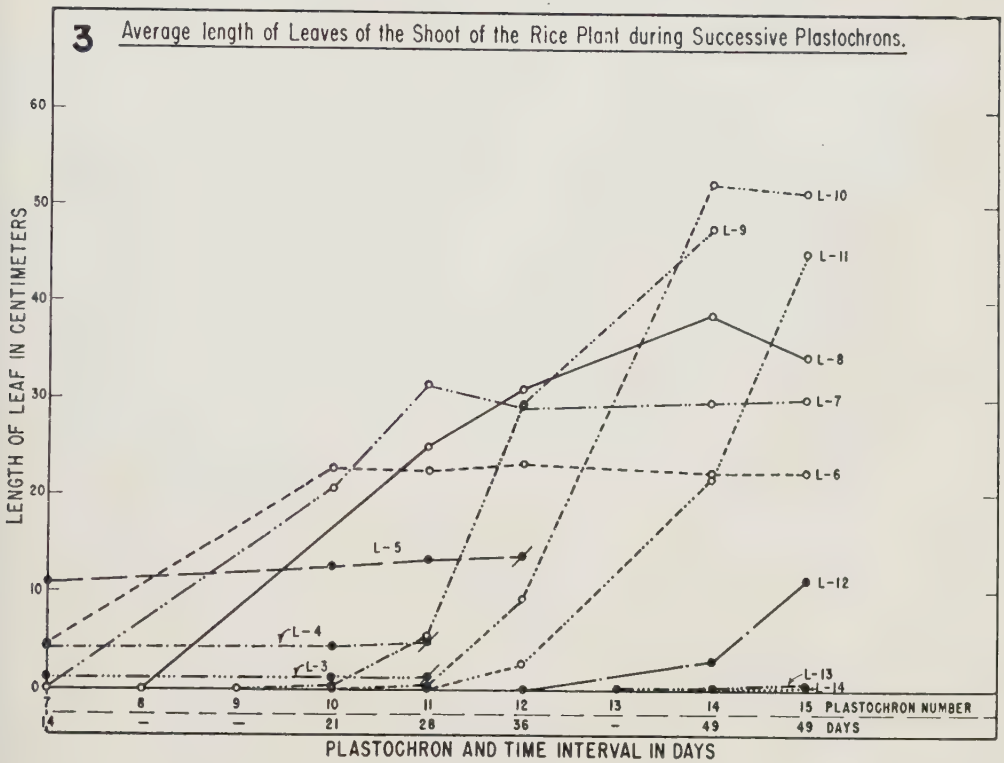
In order to obtain a clearer picture of data presented in Table 1, graphs 1-3 were prepared. These illustrate only trends in leaf development because, obviously, the number of plants used for the measurements was not adequate for statistical treatment. They reveal that in the leaves developing later (plastochrons 8 to 12),

←—

FIGS. 9, 10—(*AMP*, armed mesophyll parenchyma; *BC*, bulliform cells; *CB*, commissural bundle; *D*, diaphragm; *IS*, intercellular spaces; *L*, lacunae; *LA*, lamina; *LA-p*, paradermal section of lamina; *LEC*, long epidermal cell; *LVB*, longitudinal vascular bundle; *MP*, mesophyll parenchyma; *P*, epidermal papillae; *S*, stomata in Fig. 9, portions of sheath in Fig. 10; *ST*, stomata in Fig 10; *T*, unicellular and two-celled trichomes; *VT*, vascular tissue). Both. × 320. Fig. 9. Paradermal section near adaxial surface of lamina. Fig. 10. L.s. shoot cut parallel to the planes of the leaf blades. It includes paradermal section of lamina of younger leaf in center, longitudinal section of two portions of lamina of older leaf at left and right of center lamina section, and two portions of sheath from a still older leaf at left and right of the lamina.


1 Average Length of Lamina of the Shoot of the Rice Plant during Successive Plastochrons.
LENGTH OF LAMINA IN CENTIMETERS
60
50
40
30
20
10
0
L-10
L-9
L-11
L-8
L-7
L-6
L-12
L-5
L-4
L-3
L-13
L-14
8 9 10 11 12 13 14 15 PLASTOCHRON NUMBER
14 – – 21 28 36 – 49 49 DAYS
PLASTOCHRON AND TIME INTERVAL IN DAYS


2 Average Length of Sheaths of the Shoot of the Rice Plant during Successive Plastochrons.
LENGTH OF SHEATH IN CENTIMETERS
60
50
40
30
20
10
0
L-10
L-9
L-12
L-11
L-8
L-7
L-6
L-5
L-4
L-3
L-13
8 9 10 11 12 13 14 15 PLASTOCHRON NUMBER
14 – – 21 28 36 – 49 49 DAYS
PLASTOCHRON AND TIME INTERVAL IN DAYS



with few exceptions, the most rapid rate of elongation of lamina, sheath, and total leaf appears to occur about three plastochrons after the initiation of these leaves or parts of these leaves. When comparing curves for laminae and sheaths, one observes that the sheath is consistently initiated one plastochron *later* than the lamina. The anatomical data support this evidence. One of the most important facts revealed by the curves is that the most rapid rate of elongation of the lamina precedes that of the sheath by one or two plastochrons (leaf 10 represents an exception). This observation agrees with the previously mentioned histologic evidence of basipetal progress of leaf elongation.

Initiation and Development of the Leaf Primordium

At any given time vegetative and transitional shoots have only one primordial leaf protuberance (a primordial leaf protuberance in rice is a crescent-shaped leaf primordium which does not yet exhibit dorsiventral symmetry) immediately subjacent to the apical meristem (Figs. 2-4).[2] Each leaf primordium arises from the shoot apex as a lateral protuberance, opposite and above the last-formed primordium, extending outward to form an angle of about 90° from the main axis (Figs. 7, 8).[2] This protuberance then begins to extend laterally around the shoot apex, forming a crescent-shaped structure (Fig. 4).[2] It is thickest at the point of origin and tapers toward the two margins. After this stage the primordium begins to grow vertically (Figs. 2, 7, 9, 10).[2] It thus becomes an organ possessing dorsiventral symmetry. As the leaf primordium elongates, it forms a hood over the shoot apex (Figs. 1, 4)[2], but the margins

2. Refers to figures presented in paper I on the shoot apex of *Oryza* (Kaufman, 1959).

TABLE 1 — LENGTHS OF LAMINAE AND SHEATHS OF LEAVES OF RICE PLANTS DURING PLASTOCHRONS 7, 9-12, 14 AND 15

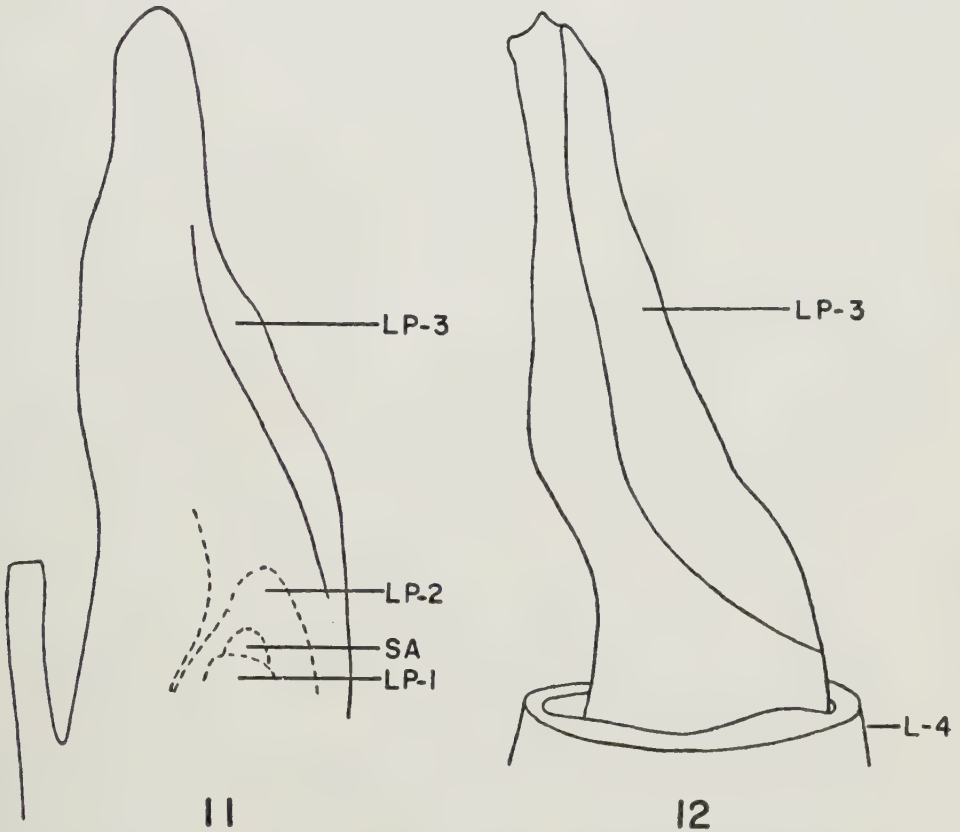
Leaf number (oldest to youngest)	Part of leaf measured	Average* lengths (cm) during the various plastochrons**						
		7	9	10	11	12	14	15
3	sheath	—	—	—	—	—	—	—
	lamina	1·3	1·4	1·1	1·2	—	—	—
	total leaf	1·3	1·4	1·1	1·2	S	S	S
4	sheath	3·5-L	4·3-L	3·2-L	3·5-L	—	—	—
	lamina	1·1	1·2	1·1	1·4	—	—	—
	total leaf	4·6	5·5	4·3	4·9	S	S	S
5	sheath	4·1-L	7·2-L	6·3-L	6·2-L	6·5	—	—
	lamina	6·6	9·2	6·3	7·2	7·2	—	—
	total leaf	10·7	16·4	12·6	13·4	13·7	S	S
6	sheath	0·1	6·8-L	9·8-L	9·5-L	9·8	8·5	9·4
	lamina	4·4	15·2	12·9	12·9	13·3	12·3	12·8
	total leaf	4·5	22·0	22·7	22·4	23·1	20·8	22·2
7	sheath	—	0·1	4·0-L	13·9-L	12·4	12·8	12·6
	lamina	—	6·1	16·9	17·6	16·7	16·7	17·1
	total leaf	LP	6·2	20·9	31·5	29·1	29·5	29·7
8	sheath	—	—	0·1	4·8-L	15·6	16·5	14·7
	lamina	—	—	3·4	20·1	15·4	22·1	19·5
	total leaf	—	0·3 (LP)	3·5	24·9	31·0	38·6	34·2
9	sheath	—	—	—	0·9	7·8	19·4	18·2
	lamina	—	—	—	4·6	21·6	28·3	—
	total leaf	—	LP	0·11 (LP)	5·5	29·4	47·7	—
10	sheath	—	—	—	—	0·9	19·4	20·0
	lamina	—	—	—	—	8·3	32·9	31·4
	total leaf	—	—	LP	0·15 (LP)	9·2	52·3	51·4
11	sheath	—	—	—	—	—	0·5	17·3
	lamina	—	—	—	—	—	21·2	27·8
	total leaf	—	—	—	LP	0·78 (LP)	21·7	45·1
12	sheath	—	—	—	—	—	0·1	0·3
	lamina	—	—	—	—	—	2·5	10·9
	total leaf	—	—	—	—	LP	2·6	11·2
13	sheath	—	—	—	—	—	—	—
	lamina	—	—	—	—	—	—	—
	total leaf	—	—	—	—	—	0·1 (LP)	0·9 (LP)
14	sheath	—	—	—	—	—	—	—
	lamina	—	—	—	—	—	—	—
	total leaf	—	—	—	—	—	LP	LP
15	sheath	—	—	—	—	—	—	—
	lamina	—	—	—	—	—	—	—
	total leaf	—	—	—	—	—	—	LP

*The averages were obtained from the following numbers of plants for the successive plastochrons numbered 7, 9-12, 14 and 15: 9, 4, 6, 10, 5, 6, 3. Complete data in original thesis are on file in the Library, University of California, Berkeley and Davis, California.

**The ages of the plants in days during the successive plastochrons numbered 7, 9-12, 14 and 15 were as follows: 14, 21, 21, 28, 36, 49, 49.

of the leaf primordium do not overlap. This stage marks the end of the first plastochron, for as the leaf primordium overtops the apical meristem, a new primordial leaf protuberance appears at the side of the apical meristem opposite and above the locus of origin of the next lower leaf (Figs. 4, 7).[2] After the hood stage the margins of the leaf primordium begin to overlap, and apical growth elicits longitudinal extension of the primordium. At this stage (plastochron 2) the primordium appears as an irregularly shaped cone, attenuated for a considerable distance from the tip, and is relatively broad at the base (Figs. 11, 12). Early in the third plastochron a ligule is initiated from the adaxial surface of the leaf primordium. With the inception of the ligule the primordium is here regarded as having entered the young leaf stage of development (*see* p. 277 for definition of young leaf). Just after ligule initiation the young leaf has a lamina many times longer than the short basal segment which represents the sheath (applies to all leaves except 1 to 4 in the rice shoot; cf. Table 1).

Leaf initation is marked by an increase in frequency of anticlinal divisions and density of cytoplasmic staining in *T*-1 along the lower flank of the apical meristem. Following this activity one to several periclinal divisions appear in *T*-1 and/or *T*-2 of the tunica (*T*-1 where one



FIGS. 11, 12 — (*LP*-1, *LP*-2, *LP*-3, leaf primordia 1, 2 and 3; *L*-4, base of leaf 4; *SA*, shoot apex). Three dimensional drawings of leaf primordia as seen in shoots of living plants where older leaves have been excised. *LP*-1 is crescent-shaped and represents plastochron 1 stage. *LP*-2 is hood or cowl-shaped and in plastochron 2 stage of development. *LP*-3 is a conical structure and in plastochron 3 stage. The base of leaf 4 is depicted in Fig. 12. Fig. 11. × 90; Fig. 12. × 95.

layer is present). Some of these features are depicted in Figs. 7, 11.[2] A lateral protuberance at the flank of the apical meristem is thus formed. Continued anticlinal and periclinal divisions in *T*-1 or *T*-2 contribute to continued enlargement of the protuberance (Figs. 3, 8).[2] The significant feature here is this: *T*-1 of the apical meristem not only produces the protoderm, but also a portion of the internal tissue of the leaf primordium. *T*-2 and peripheral regions of the corpus also contribute to the formation of this internal tissue (Figs. 2, 6, 9).[2]

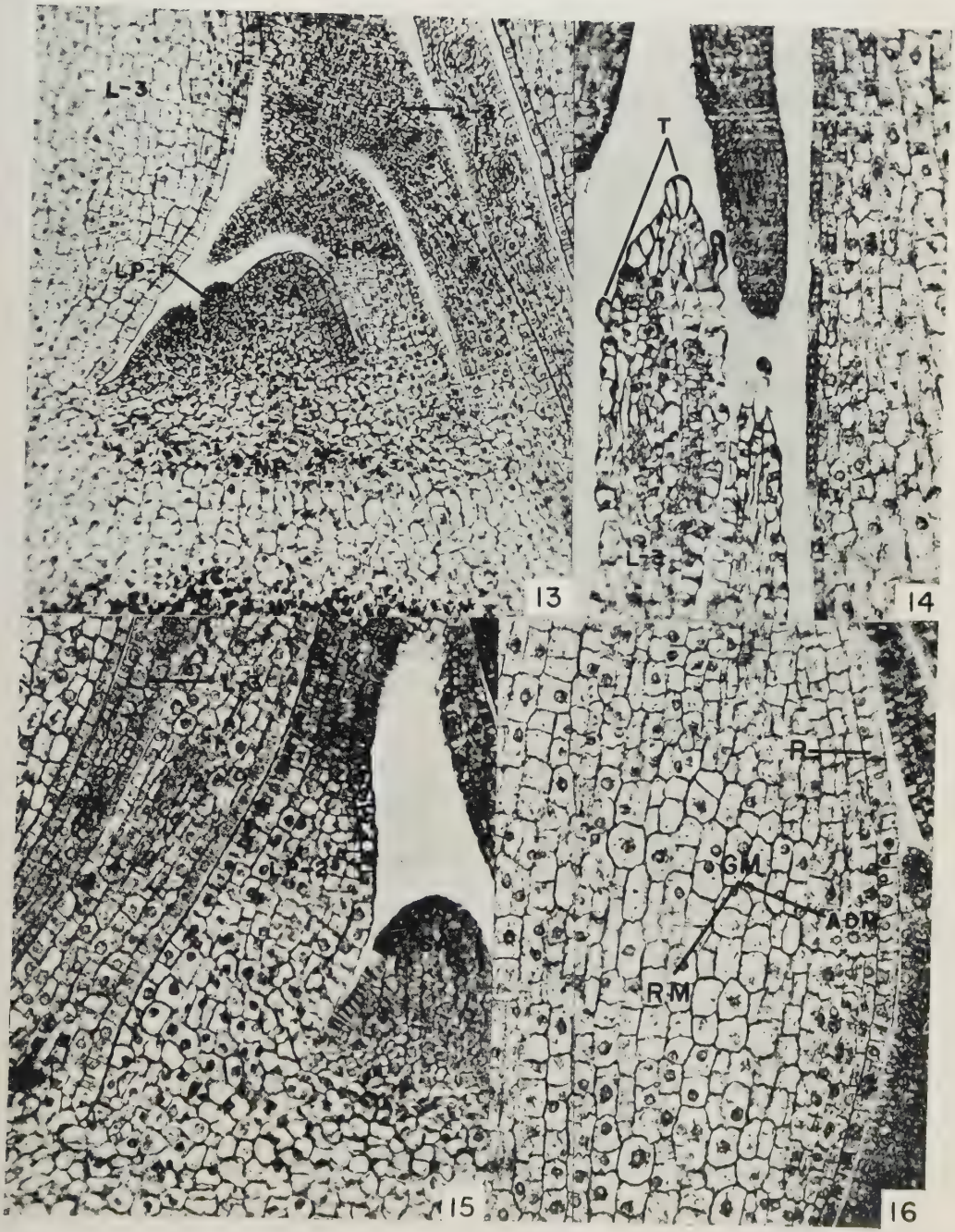
Apical growth of the leaf primordium is initiated during plastochron 2, as the primordium assumes dorsiventral symmetry and before it is 0·3 mm high, and continues through plastochron 3. This is in close agreement with data of Sontag (1887) for other grasses. During active apical growth, cells of the primordium divide repeatedly in an anticlinal direction (Figs. 13, 15). Protoderm cells at the tip of the primordium are not morphologically distinct from their derivatives and, therefore, no specific apical cells were recognized. No periclinal divisions were observed in the apical protoderm cells. After cessation of active apical growth a number of the apical protoderm cells develop into trichomes (Fig. 14). Subjacent to the protoderm of the leaf primordium short columns of cells, which mark early stages of rib meristem activity, become evident during active apical growth (Fig. 16). Apical growth ceases during the third plastochron when the leaf primordium is approximately 0·9 mm high. Termination of apical growth is usually manifest by cell enlargement and increase in vacuolation of cytoplasm in the upper part of the leaf primordium (*L*-3 in Fig. 14).

Early elongation of the primordium is elicited by cell division throughout the body of the primordium. During this stage, elongation is not confined to any one region of the primordium. After apical growth ceases, cells at the tip of the primordium cease dividing and begin to enlarge. This enlargement also contributes to elongation of the primordium but not in the same way as the rib meristem in which cell division — in a plane perpendicular to the long axis of the leaf — predominates. After cessation of cell enlargement at the tip of the primordium, rib meristem activity is primarily responsible for continued elongation of the primordium.

In the leaf primordium marginal meristem activity (Figs. 17-21) is similar to and is continuous with that of the lamina (cf. Figs. 21, 22). It is interesting to note the similarity of this pattern to that in *Zea mays* (Mericle, 1950). Mericle, however, did not indicate whether his scheme referred to the lamina or leaf primordium. Since most of the leaf primordium ultimately becomes lamina in the rice plant, the similarity and continuity of the marginal meristems of lamina and leaf primordium might be expected. No periclinal divisions were observed in marginal initials of leaf primordia or in the protoderm extending from the margin of the wings to the midrib of the primordium.

→

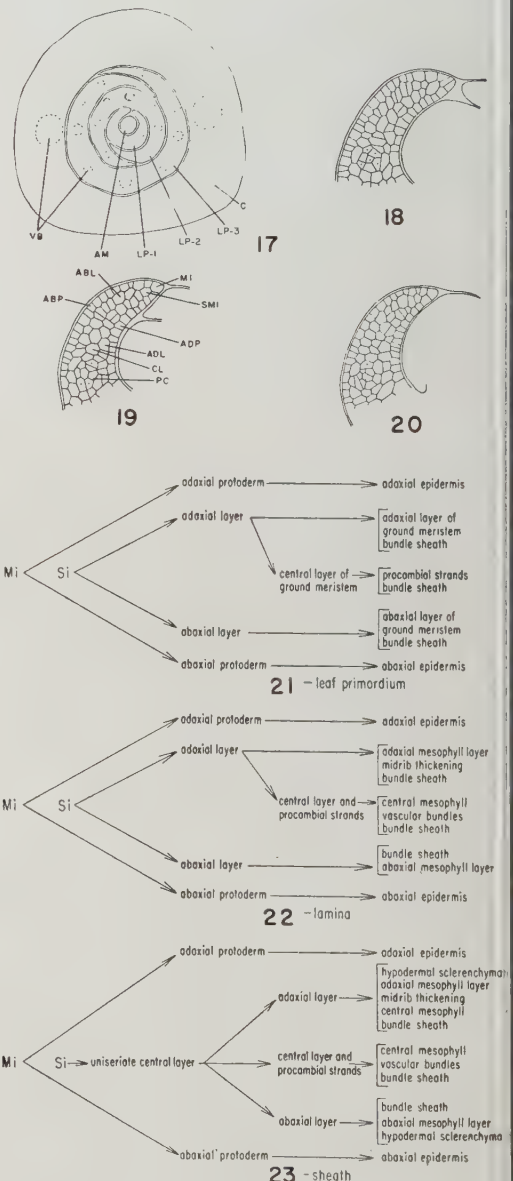
Figs. 13-16 — (*ADM*, adaxial meristem; *GM*, ground meristem; *L*-3, leaf 3; *LP*-1, *LP*-2, leaf primordia 1 and 2; *NP*, nodel plate; *P*, protoderm; *RM*, rib meristem; *SA*, shoot apex; *T*, young trichomes). Fig. 13. Median l.s. of shoot cut in plane perpendicular to the planes of the leaf blades. Protoderm and ground meristem of *LP*-2 are cytohistologically similar; the ground meristem is not yet organized into a rib meristem. The nodal plate occurs at insertion region of leaf 3. × 250. Fig. 14. L.s. through apical region of leaf 3. Cells of protoderm which have enlarged into bulbous protuberances are young trichomes. Apical growth in leaf 3 has practically ceased as evidenced by highly vacuolate cells in apical region; these cells are also larger than ground meristem cells at lower levels of leaf 3 (not seen in photograph). × 450. Fig. 15. L.s. of portion of shoot, illustrating differentiation of distinct protoderm and ground meristem tissues in leaf primordium 2. Ground meristem tissue in this leaf is not yet organized into a rib meristem. × 450. Fig. 16. Median l.s. through third leaf from shoot apex at about same level as in Fig 15. At this stage of development ground meristem tissue in this leaf has formed rib and adaxial meristems. Products of repeated periclinal divisions occur in adaxial meristem at right; uniseriate columns of cells of rib meristem are predominantly in the two-cell stage. × 450.



FIGS. 13-16.

The present observations on apical and marginal growth in the leaf primordium corroborate statements of Esau (1953) on this subject: that apical and marginal growth in the grass leaf are not distinct processes as in the dicotyledonous leaf; and that the grass leaf does not have two separate stages of development, namely, midrib-petiole leaf axis formation and lamina development, which are found in the dicotyledonous leaves that have been investigated.

During the first plastochron, cells of the ground meristem in the leaf primordium appear more or less isodiametric and possess fairly homogeneous cytoplasm. In the early stages of plastochron 1, cells subjacent to the abaxial protoderm divide more rapidly in the anticlinal plane than those near the adaxial protoderm; this brings about dorsiventral symmetry of the leaf primordium. Then, in early stages of plastochron 2, some cells of the ground meristem in the central and basal part of the leaf primordium become conspicuously longer than wide because of vertical divisions. This marks the locus of initiation of the median procambial strand. Examination of serial transverse and longitudinal sections of leaf primordia (Kaufman, 1954, Figs. 54-60) revealed that the median procambial strand of the leaf primordium differentiates acropetally in the leaf primordium during the second plastochron. Four lateral procambial strands are initiated in the leaf primordium late in the same plastochron and also differentiate acropetally. Moreover, both protophloem and protoxylem in these strands differentiate acropetally. The protophloem differentiates before the protoxylem. During plastochrons 2 and 3, a rib meristem (Fig. 16) and an adaxial meristem (Figs. 16, 24-27) are

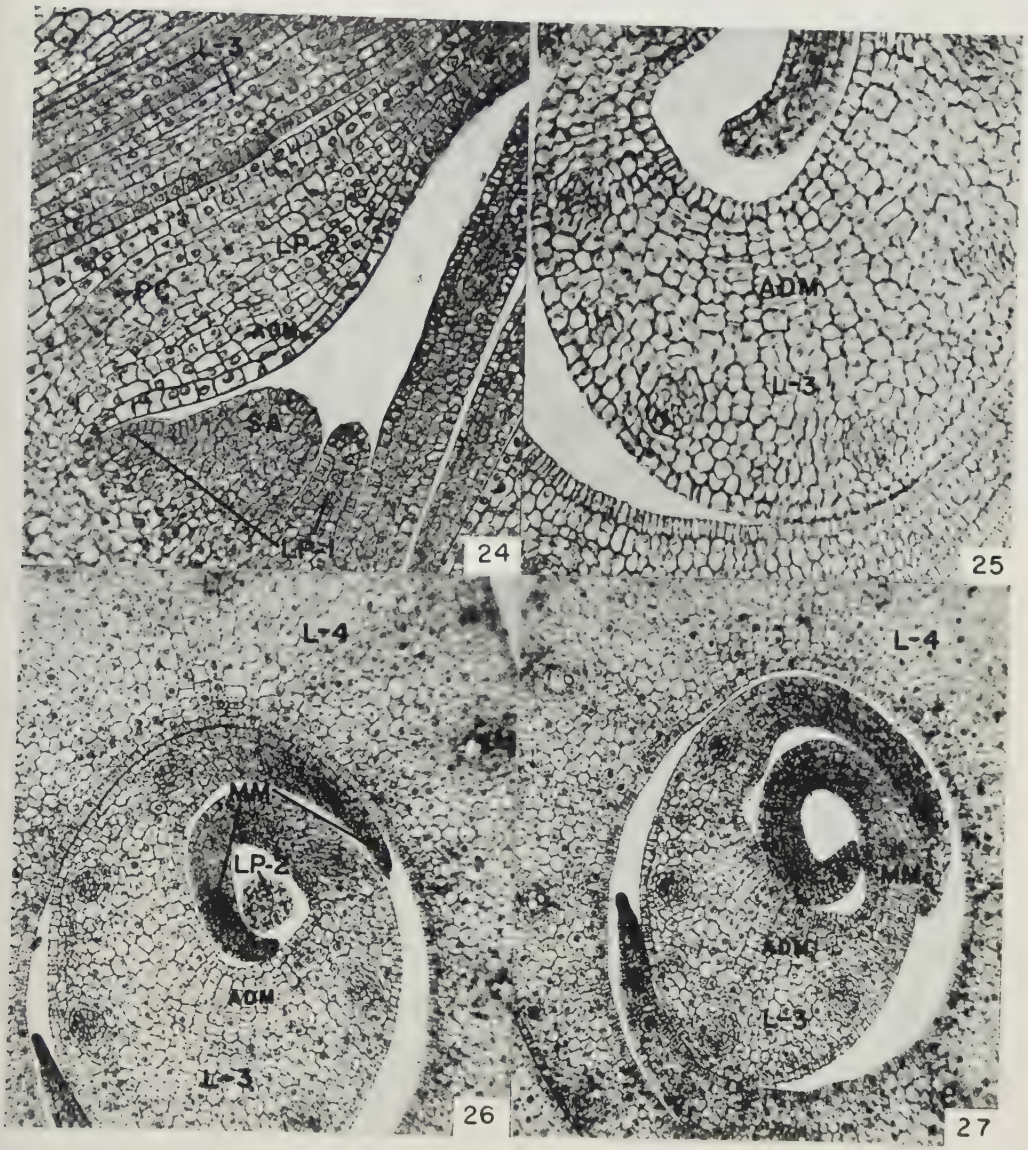


FIGS. 17-23.

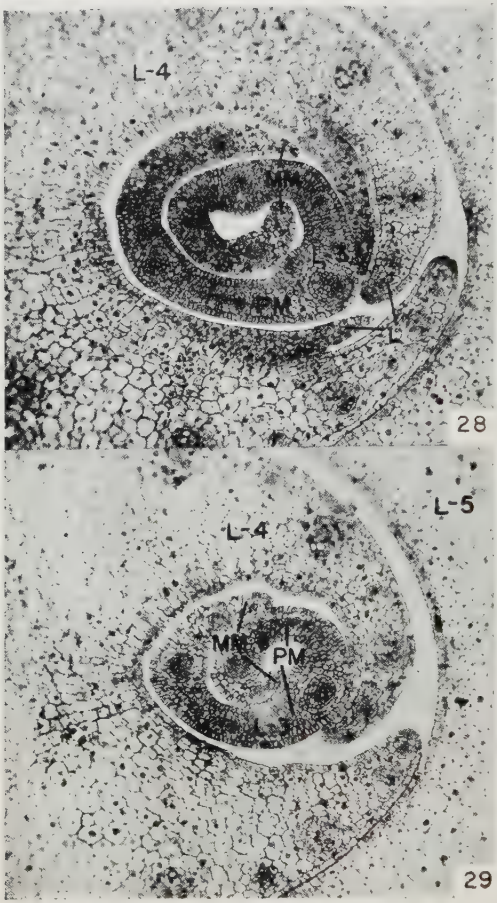
FIGS. 17-23 —(*ABL*, abaxial layer; *ABP*, abaxial protoderm; *ADL*, adaxial layer; *ADP*, adaxial protoderm; *AM*, apical meristem; *C*, coleoptile; *CL*, central layer; *LP*-1, *LP*-2, *LP*-3, leaf primordia 1, 2 and 3; *MI*, marginal initial; *PC*, procambial strand, which includes dotted cells; *SMI*, submarginal initial; *VB*, vascular bundles). Fig. 17. T.s. through shoot of three-day old seedling. × 60. Figs. 18-20. Drawings depicting one of marginal meristems of *LP*-3 of Fig. 17 at following levels above that of the leaf insertion region: 5μ in Fig. 18; 15μ in Fig. 19; 45μ in Fig. 20. Figs. 18-20. × 250. Figs. 21-23. Diagrams depicting growth patterns in the marginal meristem of the wings of the leaf primordium (Fig. 21), lamina (Fig. 22) and sheath (Fig. 23). Descriptive details for these diagrams are found in the text.

organized in the future midrib, and marginal and plate meristems (Figs. 26-29) in the wings.

The protoderm of the leaf primordium is a uniseriate layer which becomes distinct soon after the primordium is initiated in



FIGS. 24-27 — (*ADM*, adaxial meristem; *AM*, apical meristem; *L*-3, *L*-4, leaves 3 and 4; *LP*-1, *LP*-2, leaf primordia 1 and 2; *MM*, marginal meristems; *PC*, procambial strand). Fig. 24. Median l.s. of leaf primordium 2, illustrating adaxial and abaxial protoderms, adaxial and ground meristems. × 450. Fig. 25. T.s. of leaf 3, depicting the radial series of cells (center of photograph) constituting the adaxial meristem; this seriation is a consequence of repeated periclinal divisions in each radial row of cells. × 450. Fig. 26. T.s. of shoot at level that passes through tip of leaf primordium 2 and basal region (future sheath) of leaf 3. × 260. Fig. 27. T.s. of same shoot as in Fig. 26 except at a higher level. In leaf 3 (future lamina base at this level) adaxial meristem activity is less apparent than at lower levels. There is some evidence of plate meristem activity in each of the wings. × 260.



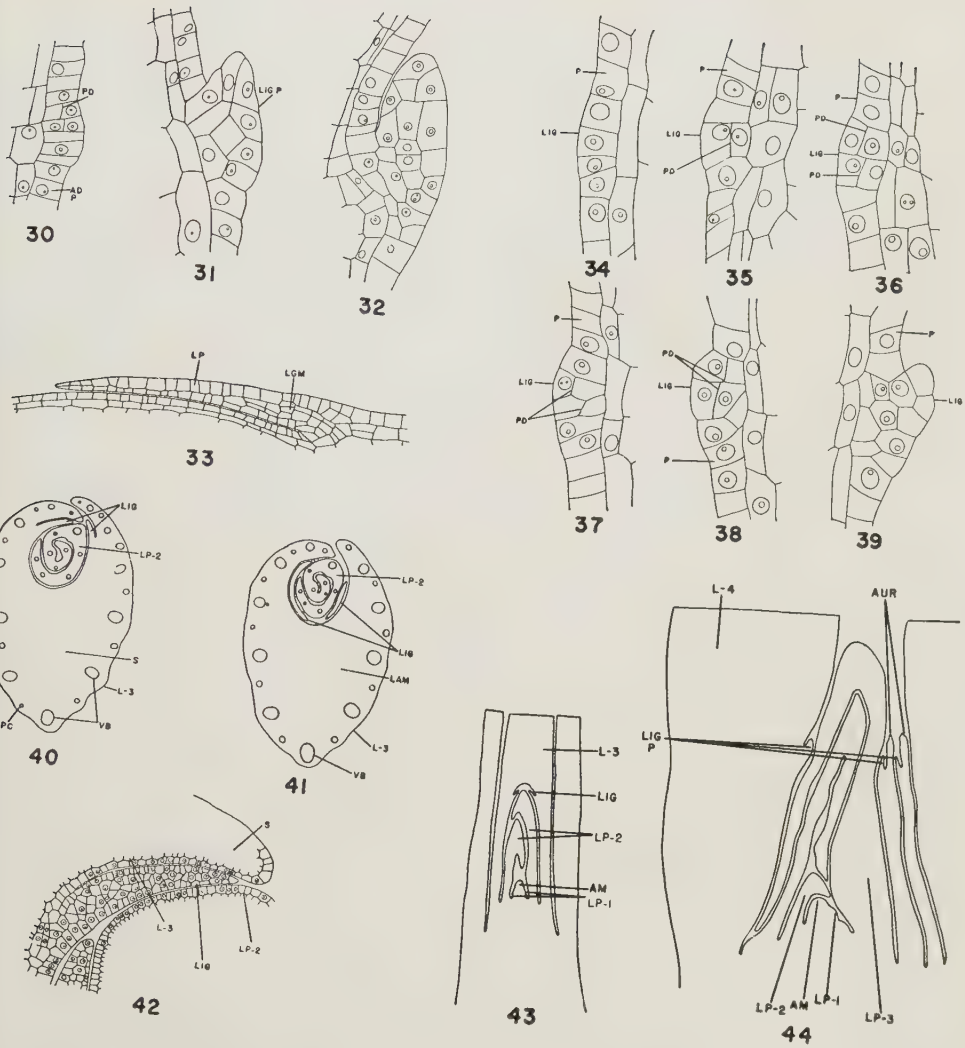
FIGS. 28, 29 — (*L*, ligule; *L*-3, *L*-4, *L*-5, leaves 3, 4 and 5; *MM*, marginal meristems; *PM*, plate meristem). Both. × 250. Fig. 28. T.s. of same shoot as in Figs. 26, 27 except at higher level. Leaf 3 at this level represents central portion of future lamina. The midrib is devoid of an adaxial meristem; prominent plate and marginal meristems occur in the wings. Fig. 29. T.s. of same shoot as in Fig. 28 except at higher level. Leaf 3 at this level is represented by portion near the tip. A distinct midrib is absent.

the apical meristem. The uniformity of this layer is only interrupted by the initiation of trichomes at the apex of the primordium (plastochron 2) and ligule and auricles between future lamina and sheath regions (plastochron 3). No periclinal divisions were identified in apical or marginal initials of leaf primordia. During plastochrons 1, 2 and 3 protoderm cells at the tip of the primordium enlarge considerably and become progressively more vacuolate. By the end of plastochron 3, trichomes and stomata initials can be detected in the protoderm in the uppermost portion of a primordium.

The Formation of Ligule and Auricles

According to the present interpretation, the initiation of ligule and auricles marks the termination of primordial leaf development and the beginning of differentiation of lamina and sheath. In the rice plant the ligule and auricles are initiated during the third plastochron. This process is first indicated by the appearance of one to several periclinal divisions in the adaxial protoderm of the leaf primordium (Fig. 30). As the cells resulting from these divisions enlarge and continue to divide, a protuberance becomes apparent (Figs. 30-32). Early in development this structure is oriented in a plane perpendicular to the longitudinal axis of the leaf. With continued growth the ligule assumes dorsiventral symmetry (cf. Figs. 30-32, 45). Initially the ligule is a small localized adaxial protuberance. By means of continued periclinal divisions in protoderm cells the ligule enlarges laterally toward the margins of the sheath wings (Figs. 34-39). The ligule cells are derived exclusively from the adaxial protoderm of the leaf primordium. This observation is in agreement with findings of Philipson (1935) and Neumann (1937).

As the ligule assumes dorsiventral symmetry, cells of the ground meristem of the ligule become conspicuously vacuolate and divide predominantly in an anticlinal direction. Concomitantly, there is considerable apical growth which ultimately leads to the formation of a long biseriate segment in the upper region of the ligule (Fig. 33). Cells of these two series continue to divide anticlinally after cessation of apical growth and ultimately form the adaxial and abaxial protoderms. The internal tissue of the ligule increases in extent as a result of cell division in subapical cells and lineal descendants of these cells (Figs. 33, 42). The position and

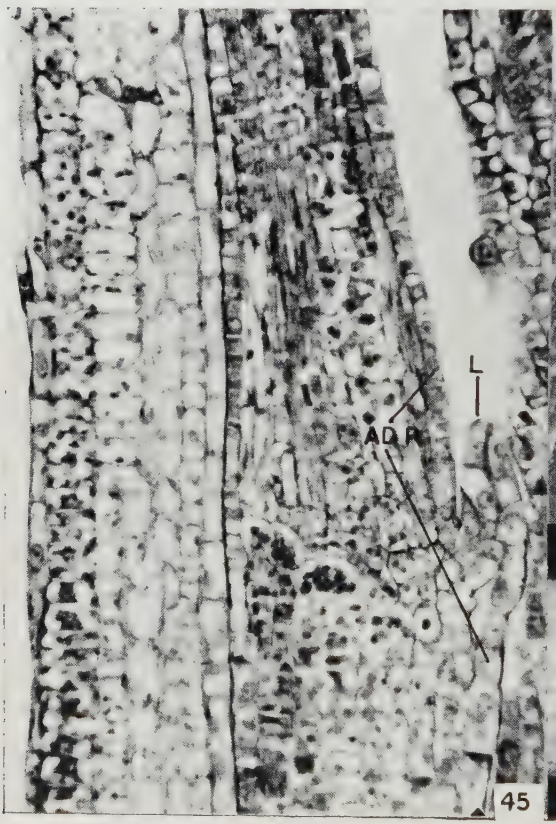


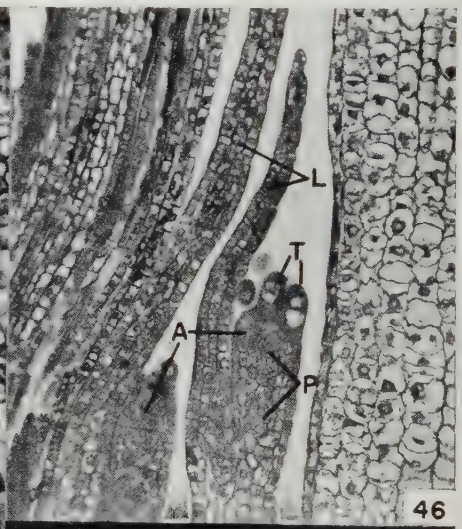
FIGS. 30-44 — (*ADP*, adaxial protoderm; *AM*, apical meristem; *AUR*, auricles; *L*-3, *L*-4, leaves 3 and 4; *LAM*, lamina; *LGM*, ligule ground meristem; *LIG*, ligule; *LIG P*, ligule protuberance; *LP*, ligule protoderm; *LP*-1, *LP*-2, *LP*-3, leaf primordia 1, 2 and 3; *P*, sheath protoderm; *PC*, procambial strands; *PD*, periclinal divisions; *S*, sheath; *VB*, vascular bundles). Figs. 30-33. Median l.s. of ligules at different stages of development. Fig. 30. Periclinal divisions in sheath adaxial protoderm mark ligule initiation. × 760. Fig. 31. Ligule protuberance exhibits dorsiventral symmetry at this stage. × 760. Fig. 32. Ligule at later stage of development. × 760. Fig. 33. Ligule after much elongation has occurred. Some internal ground meristem and a protoderm are apparent. × 170. Figs. 34-39. Series of l.s. of a single ligule at different positions of attachment to sheath. All. × 700. Fig. 34. Margin of ligule. Figs. 35-38. Series of sections between margin and center of ligule. Fig. 39. Section through median plane of attachment (center) of ligule. Figs. 40-42. T.s. through shoot in region of attachment of ligule to leaf 3 from apical meristem. Fig. 40. Section at level of sheath-lamina transition region. × 75. Fig. 41. Section 573 μ above section in Fig. 40 at level of lamina base of leaf 3. × 75. Fig. 42. Some cellular details of ligule attached to sheath of leaf 3. Marginal growth pattern of ligule is similar to that of the sheath. × 170. Figs. 43, 44. Median l.s. depicting position of ligule and auricles at early stage of development in the shoot. Fig. 43. Section in plane parallel with the planes of the leaf blades, showing two protuberances (wings of ligule) arising from third leaf from apical meristem. × 60. Fig. 44. Section in plane perpendicular to the planes of the leaf blades. Ligule protuberance and two auricles shown are associated with leaf 4 from shoot apex. × 90.

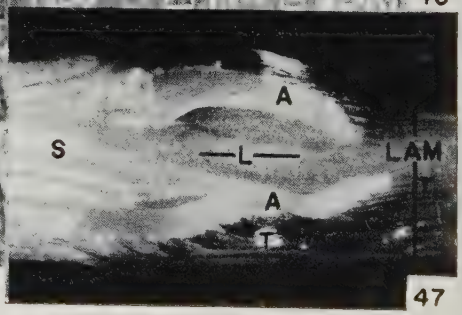
form of mature ligules are depicted in Figs. 40, 41, 43, 44, 47.

The auricles of the rice plant are two sickle-shaped appendages which are attached to the margins of the leaf where the ligule joins the uppermost part of the sheath. They are directly connected to both sheath and ligule. The young auricles are oriented vertically (Fig. 47), but after the leaf emerges from the outer leaves and the ligule becomes exposed, the auricles encompass the sheath of the next inner leaf in a horizontal direction.

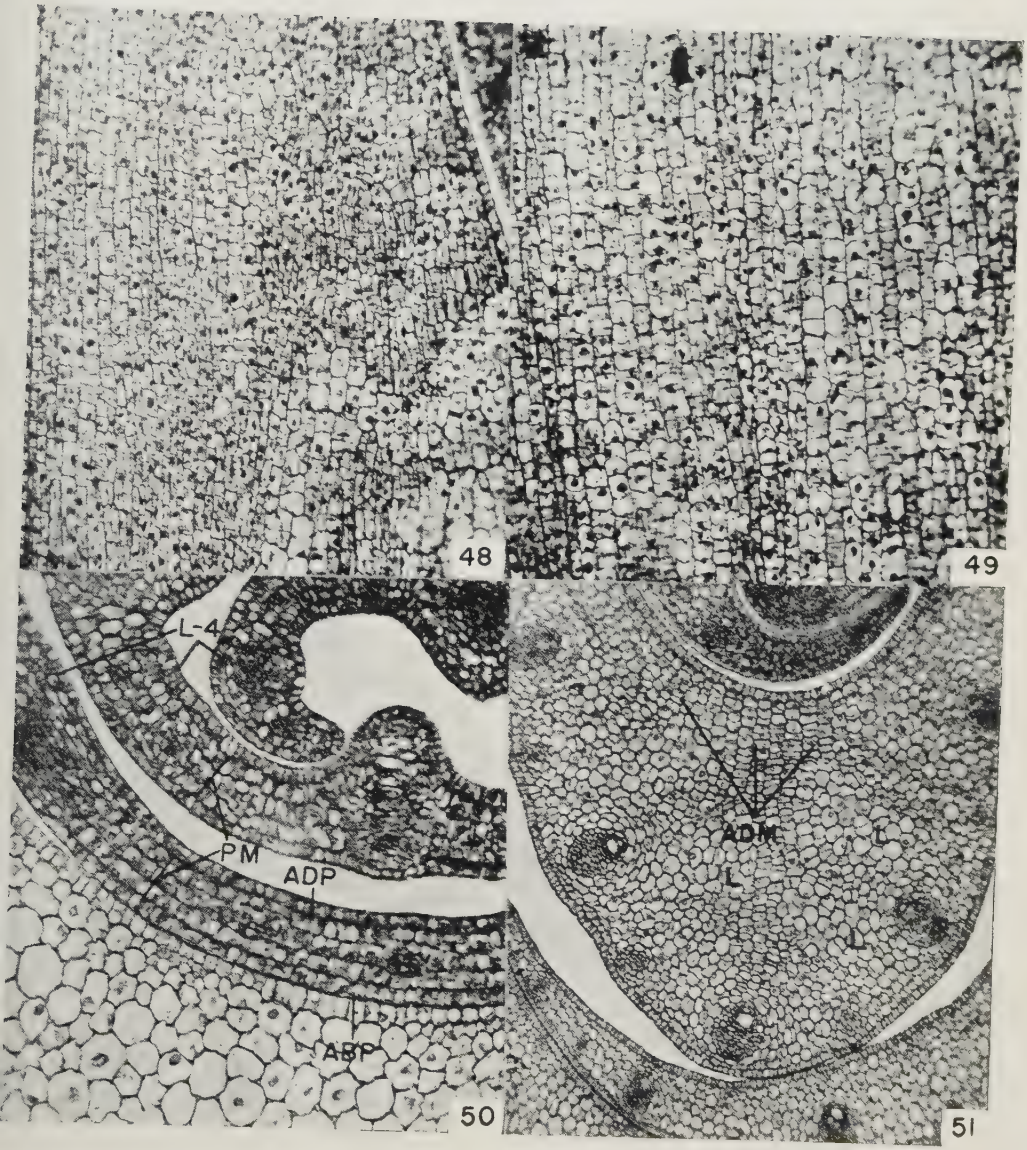
Auricle primordia appear to be derived from both the protoderm and ground meristem of sheath and ligule. In recently initiated auricle primordia the protoderm of the auricle, sheath and ligule is continuous, and the ground meristem of the sheath extends into the auricle (Fig. 46). At the apex of a young elongating auricle, protoderm cells begin to enlarge; these are young trichomes (Fig. 46). During successive stages of development they become globular, then elongated, and ultimately form greatly attenuated trichomes (Fig. 47). Nuclei and cytoplasm were observed in many of the elongating trichomes (Fig. 94). Trichomes also develop along the abaxial margin of the auricles (Fig. 47).







FIGS. 45-47 — (*A*, auricle; *ADP*, adaxial protoderm; *L*, ligule; *LAM*, lamina; *P*, two procambial strands; *S*, sheath; *T*, trichomes). Fig. 45. Median l.s. of young ligule and adjacent leaf parts. × 740. Fig. 46. T.s. of one auricle and part of another; l.s. through shoot axis. The auricle is attached to the sheath and margin of the ligule. × 400. Fig. 47. View of two auricles and ligule attached to a young leaf. Auricles have vertical position at this stage; later, they become horizontal. × 10.



FIGS. 48-51 — (*ABP*, abaxial protoderm; *ADM*, adaxial meristem; *ADP*, adaxial protoderm; *L*, future lacuna; *L*-4, leaf 4; *PM*, plate meristems). Fig. 48. L.s. through midrib region of sheath of leaf 4 from shoot apex. Tissue composed of parallel columns of cells represents the rib meristem. Most of the columns are in two-cell stage; some cells have divided periclinally as well as anticlinally. × 250. Fig. 49. L.s. through midrib region of lamina of same leaf as in Fig. 48. At this level columns of cells composing the rib meristem are, in general, longer and the constituent cells larger than those of rib meristem in the sheath. × 250. Fig. 50. T.s. of portions of lamina wings of leaf 4 from shoot apex. Cells in the lower wing exhibit a plate-like or stratified arrangement (the three layers between the adaxial and abaxial protoderms); these constitute the plate meristem. × 450. Fig. 51. T.s. of central portion of sheath, showing last phases of adaxial meristem activity and regions (larger, more vacuolate cells) where lacunae will form. × 450.

Initiation and Development of Lamina and Sheath

Several growth patterns are manifest in the young rice leaf which can be designated as rib, plate, adaxial and intercalary meristems. They are defined according to their relative positions in the leaf and the patterns of cell division characteristic of each type. These meristems play a significant role in development of the form of the mature leaf. The most outstanding characteristics of a rib meristem are the arrangement of cells in vertical series and the repeated transverse cell divisions within these series. In longitudinal sections the constituent cells of a rib meristem occur in more or less parallel series or columns, whereas in transverse sections, the cells are arranged in an irregular manner.

In the rice leaf the parallel columns of cells seen in longitudinal sections of midribs of young laminae and central regions of sheaths are interpreted here as evidence of rib meristem form of growth. In initial stages of rib meristem activity (plastochron 3) the parallel series of cells are short, being two or three cells in length (Fig. 16). During plastochron 4 these series are much longer (Fig. 49). The rib meristem in the rice leaf is largely responsible for increase in length of the lamina midrib and central region of sheath. The adaxial meristem also plays a role in the development (growth in diameter) of these regions of the leaf.

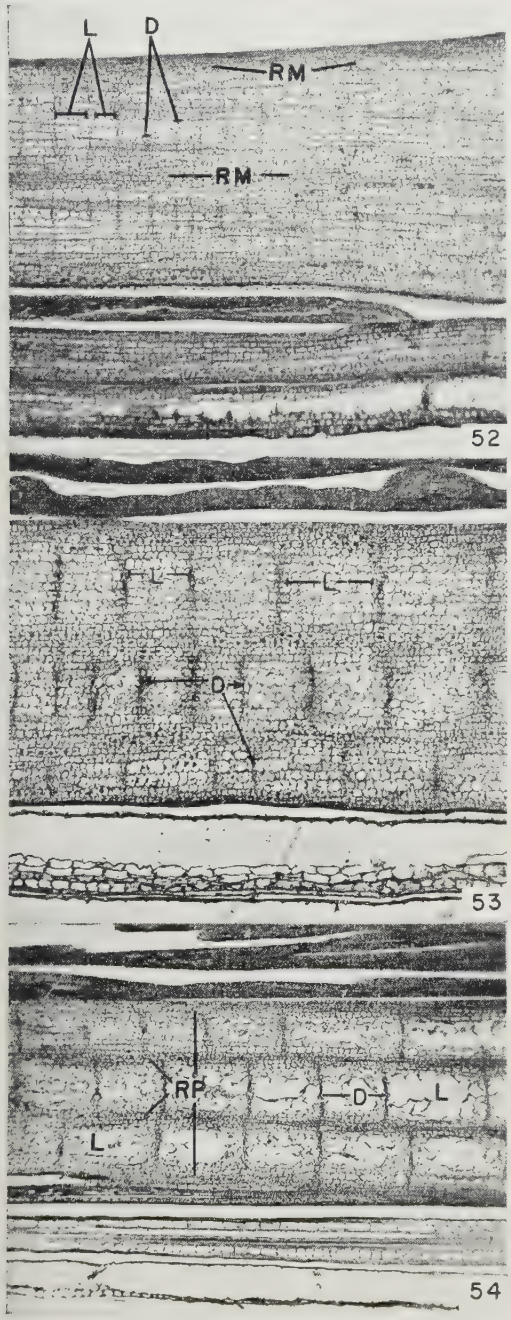
Extension of the lamina wings in the rice plant results primarily from the activity of marginal and plate meristems. Marginal growth was previously described with reference to Figs. 21 and 22. Plate meristem activity is clearly revealed in transections of young lamina wings (Figs. 50, 57, 60). It is first evident during plastochron 3 and continues through plastochron 4. At higher levels in the lamina, where no rib meristem is formed, the plate meristem appears nearly continuous from margin to margin. This accounts for the flat plate-like structure of the mature lamina. The plate meristem is regularly interrupted by procambial strands and vascular bundles, which together with adjacent ground meristem form rib-like regions in the lamina (Figs. 57, 58). The sheath wings have no distinct plate meristem zones (Figs. 56, 57). Extension of these wings is primarily due to marginal meristem activity and enlargement of the derivatives of this meristem.

An intercalary meristem, in the sense of " an actively growing primary tissue region somewhat removed from the apical meristem " (Esau, 1953), is present in the rice leaf. The time of initiation of rapid elongation of the lamina exceeds that of the sheath by one or two plastochrons. When the sheath begins to elongate actively, most of the cells and tissues of the lamina are in later stages of differentiation (cf. Figs. 52-54). Thus, we can visualize the intercalary meristem in the rice leaf as a region of actively dividing and enlarging cells — the rib and plate meristems collectively — that progressively becomes localized to the base of the sheath as the leaf develops.

As was mentioned previously, marginal growth in the rice leaf is initiated at a relatively early period in the leaf ontogeny (plastochron 2). From the time that the ligule is initiated, one can discern a distinct and regular cellular pattern in the wing of the young sheath. From the central region to the margin of the sheath the wing diminishes in thickness; in accordance, the number of cell layers, including the epidermis, decreases from many near the midrib to five, four, three, and finally two (Figs. 55-57). The triseriate and four-seriate regions are separated by a procambial strand (Fig. 55). This type of marginal pattern has also been depicted by Lund (1872; cf. Foster, 1936a). The growth pattern of the marginal meristem in the rice sheath is summarized in Fig. 23. The marginal initials of the sheath wing characteristically divide along two anticlinal facets and are responsible for the formation of a biseriate flap. The submarginal initials, located at a considerable distance from the marginal initials, divide only anticlinally, giving rise to the central layer in the triseriate region of the wing (Figs. 23, 55, 57). This central layer gives rise to the adaxial and abaxial layers (Fig. 23) and to procambial strands (Fig. 55). No plate

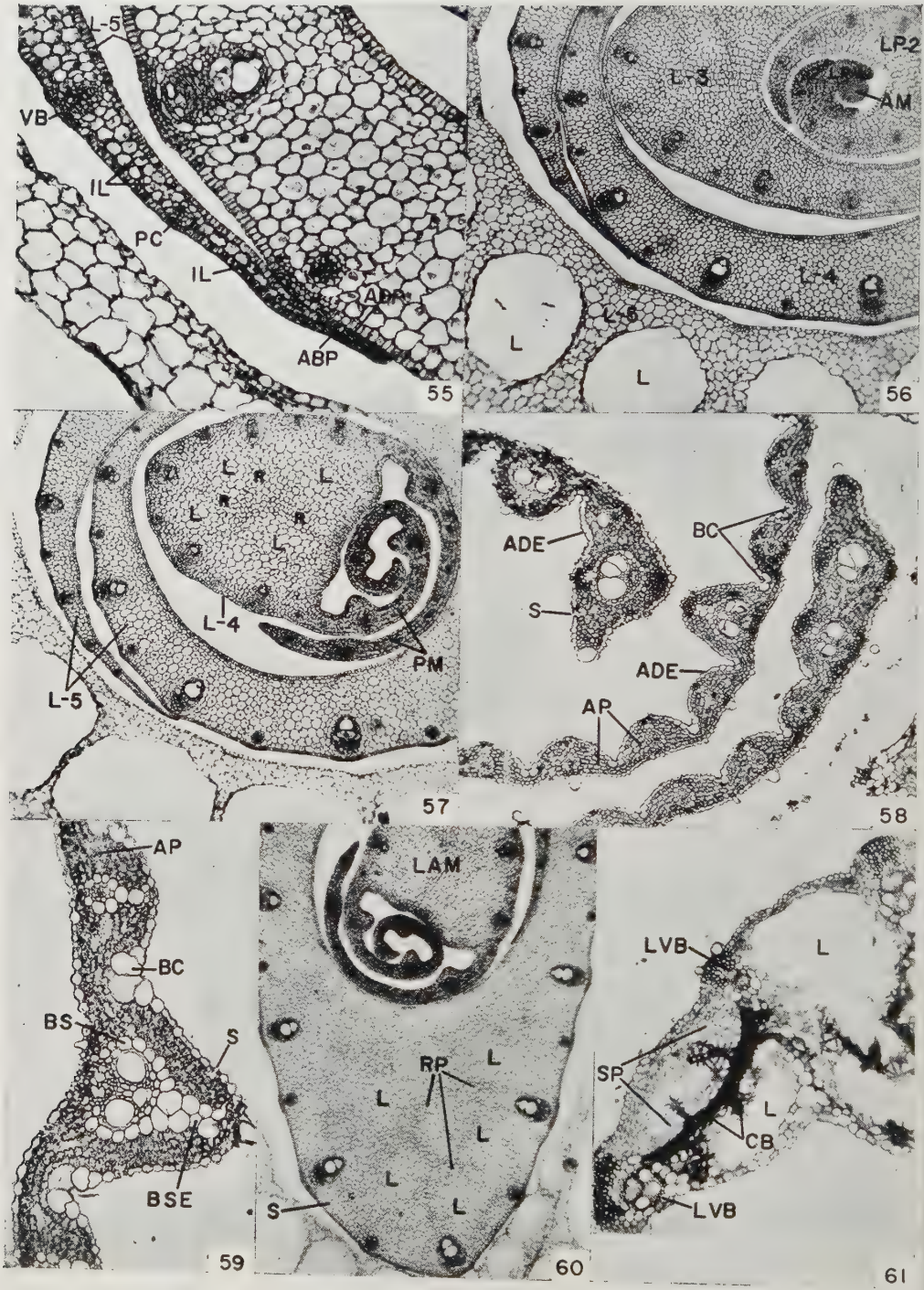
meristem is established in these internal layers of the sheath wings (Figs. 56, 57), although cells in the adaxial layer, extending the adaxial meristem activity of the central region of the sheath, may divide periclinally and contribute additional increments to the wing mesophyll parenchyma (Fig. 57).

The marginal growth pattern in the lamina is entirely different from that in the sheath (Figs. 50, 57). The base of the lamina, i.e. just above the ligule, consists of midrib only and does not exhibit marginal growth. The remainder of the lamina above this basal region has wings, which are widest about one-third of the distance from the leaf tip. Figure 22 depicts the marginal growth pattern of these wings. This growth pattern with some deviations is similar to the third type of marginal growth pattern mentioned by Gifford (1951). In some instances a central layer may be formed by oblique divisions in the adaxial layer, and cells in both adaxial and central layers contribute to the formation of procambial strands. In other instances cells of the adaxial layer are first associated with the genesis of procambial strands, and after initiation of these strands, the central layer is formed. Some procambial strands later become oriented toward the abaxial side of the lamina. This is brought about by series of periclinal divisions in the adaxial layer of the wing. The tissue interposed between the procambial strands and the adaxial protoderm later becomes the bundle sheath extension (Figs. 58, 59). Between the procambial strands in young lamina



FIGS. 52-54.

FIGS. 52-54 — (*D*, future diaphragm regions in Figs. 52, 53, lacuna diaphragms in Fig. 54; *L*, future lacunae in Figs. 52, 53, lacunae in Fig. 54; *RM*, rib meristem; *RP*, ribs of parenchymatous tissue). All. × 125. Fig. 52. L.s. through sheath midrib at level where rib meristem is still intact across entire midrib. Fig. 53. L.s. at higher level of leaf, in lamina midrib, illustrating later stage in lacuna and diaphragm development. Fig. 54. L.s. at still higher level of lamina midrib in a region where lacunae are forming. Fragments within lacunae are parts of cells of original rib meristem. Longitudinal columns of cells between lacunae, representing remnants of same rib meristem, become ribs of parenchymatous tissue between lacunae.


L-5
VB
IL
PC
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ABP
ABP
55
L-3
LP2
AM
L-4
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59
LAM
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L
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L
L
S
60
LVB
L
SP
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CB
LVB
61

FIGS. 55-61.

wings, layers of genetically related cells divide anticlinally and grow as a plate meristem (Figs. 50, 57). These cells ultimately develop into the armed parenchyma cells of the lamina mesophyll (Figs. 58, 59).

Development of Lacunae — Lacuna formation serves as a remarkably useful marker for determining the direction of maturation of the rice leaf. In the rice plant this process occurs in lamina and sheath midribs, sheath wings, stem internodes, and the cortex of the root. It is associated with the elongation of leaf, stem and root and with rib, adaxial and intercalary meristem activity.

Cells in well-defined regions of the lamina rib meristem ultimately cease dividing and begin to enlarge, becoming more vacuolate and developing conspicuous intercellular spaces (Figs. 52, 53, 57). The cell walls are apparently stretched since they become thinner. Then wall rupturing occurs, and a lacuna is formed (Figs. 52-54). The rupturing appears to be a mechanical tearing and is, therefore, interpreted here as a rhexigenous process. No evidence of enzymatic dissolution of cell walls was obtained. The process of lacuna formation is initiated during the fifth plastochron and proceeds basipetally from about the center of the lamina midrib, through the ligule region, and into the sheath central region and wings (Figs. 4, 51, 54, 60). By the end of the sixth plastochron lacunae have developed into the sheath base.

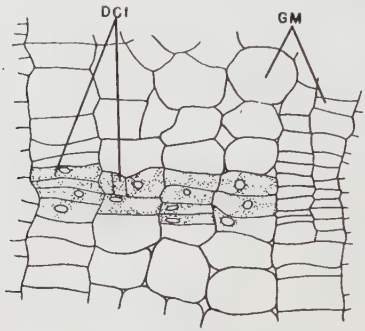
Tissue areas not involved in lacuna formation, that is areas adjacent to the adaxial and abaxial protoderms, and those in the wings and central region of the sheath and midrib of the lamina (Figs. 52-54, 57, 60) continue to divide for a time after the initiation of lacunae.

Development of Diaphragms — During plastochrons 3 and 4 uniseriate columns of cells in the rib meristem of the lamina midrib begin to elongate by means of repeated anticlinal divisions and cell enlargement. These columns do not elongate indefinitely because occasional oblique divisions in a given column initiate a new column of cells; or cells in a given column or series of columns cease dividing and begin to enlarge. This cell enlargement is first manifest in the lamina midrib and progresses basipetally into the sheath midrib and wings. In well-defined regions which intersect many uniseriate columns of cells, cells in two or three tiers do not enlarge in contrast to the ones immediately above and below (Fig. 52). These cells, which are diaphragm initials, remain densely cytoplasmic and undergo a few anticlinal and periclinal divisions without enlarging to any great extent (Fig. 62). The divisions occur during the time that lacunae are forming above and below the diaphragms. Certain diaphragm cells (tracheid initials), arranged in transverse series, divide in longitudinal and transverse planes several times (Fig. 63). The products of these divisions elongate and differentiate into tracheids.

←

Figs. 55-61 — (*ABP*, abaxial protoderm; *ADE*, adaxial epidermis; *ADP*, adaxial protoderm; *AM*, apical meristem; *AP*, armed parenchyma; *BC*, bulliform cells; *BS*, bundle sheaths; *BSE*, bundle sheath extension; *CB*, commissural bundle; *IL*, internal layer; *L*, future lacuna in Figs. 57, 60, lacunae in Figs. 56, 61; *LAM*, lamina; *L*-3, *L*-4, *L*-5, leaves 3, 4 and 5; *LP*-1, *LP*-2, leaf primordia 1 and 2; *LVB*, longitudinal vascular bundle; *PC*, procambial strand; *PM*, plate meristem; *RP*, *R*, ribs of parenchyma tissue; *S*, stomata in Figs. 58, 59, sheath in Fig. 60; *SP*, stellate parenchyma; *VB*, vascular bundle). Fig. 55. T.s. of marginal meristem and other parts of sheath of leaf 5 from apical meristem. Extreme margin is biseriate where abaxial and adaxial protoderms become confluent. × 450. Fig. 56. T.s. of successively older leaves at level of apical meristem. *L*-3, *L*-4 and *L*-5 represent sheaths. No plate meristems occur in the sheaths. × 250. Fig. 57. T.s. of leaves 4 and 5 from apical meristem, depicting sheath wings of leaf 5 and lamina midrib and wings of leaf 4. Future ribs of mesophyll tissue and lacuna regions are demarcated in the midrib of leaf 4. × 250. Figs. 58, 59. T.s. of portion of lamina of a mature leaf. Fig. 58. × 110; Fig. 59. × 450. Fig. 60. T.s. of midrib of sheath and portion of lamina of next youngest leaf. Lighter regions in the sheath represent areas where lacunae will form; darker, narrow and interconnected regions will become ribs of parenchyma tissue. × 95. Fig. 61. T.s. through portion of sheath wing, depicting two lacunae and associated diaphragms. × 95.

The divisions are confined to the original tracheid initials, the walls of the latter remaining discernible. The other diaphragm cells cease dividing earlier and begin to enlarge while tracheid initials are being formed (Figs. 63, 64). Conspicuous intercellular spaces appear among the enlarging cells. They develop between the facets of two cells or at junctions of several cells (Figs. 63, 64). Such tissue constitutes the young stellate parenchyma of the diaphragm. The fully differentiated diaphragm consists of bizarre stellate parenchyma cells, which are separated by huge sac-like intercellular spaces, and commissural bundles which join the longi-



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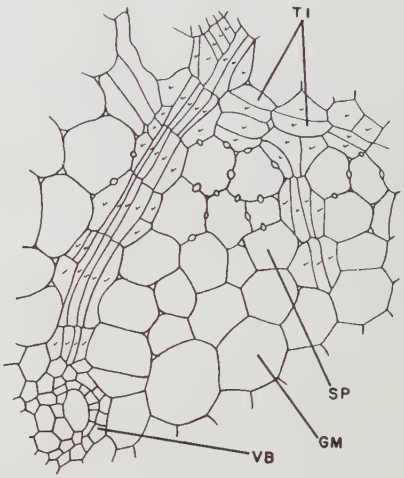

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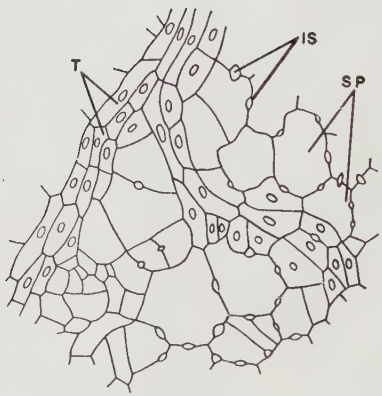

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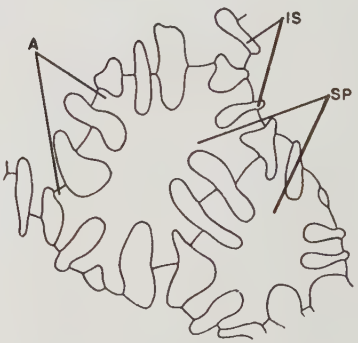

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Figs. 62-65 — (*A*, " arms " of stellate parenchyma cells; *DCI*, young diaphragm cells; *GM*, ground meristem cells; *IS*, intercellular spaces; *SP*, stellate parenchyma cells; *T*, young tracheids; *TI*, tracheid initials; *VB*, longitudinal vascular bundle). Different stages in the ontogeny of stellate parenchyma cells and tracheids of commissural bundles in lacuna diaphragms. Fig. 62. L.s. near base of sheath, depicting an early stage of diaphragm (stippled cells) development in the ground meristem. Cells represented here are products of divisons within the diaphragm initials. × 525. Fig. 63. T.s. through ground meristem in sheath midrib in locus of young diaphragm, illustrating tracheid initials (checked cells) of commissural bundles and young stellate parenchyma cells. × 475. Fig. 64. T.s. of young sheath diaphragm at slightly later stage of development. × 475. Fig. 65. Two mature stellate parenchyma cells with so-called " arms " separated by sac-like intercellular spaces. × 475.

tudinally oriented bundles located in the abaxial, adaxial and rib mesophyll (Figs. 61, 63).

Stellate parenchyma cells of the leaf diaphragm are similar to those in the pith of *Juncus* (Lewis, 1925; Geesteranus, 1941) but differ from those in the nodal diaphragms and ground tissue adjacent to internodal cavities in the stem of the rice plant (cf. Fig. 61 with Figs. 16, 17 in paper III of this series). They also differ from the armed parenchyma cells of the lamina mesophyll of the rice leaf (cf. Fig. 61 with Figs. 58, 59). These differences are attributed to variation in the loci and methods of formation of intercellular spaces.

DEVELOPMENT OF MESOPHYLL — The differentiation of various cell types in the mesophyll does not occur simultaneously. Mesophyll differentiation begins with an increase in cell size and vacuolation and is accompanied by the appearance of intercellular spaces (Fig. 57). These phenomena first occur in regions where diaphragms and lacunae are formed. They then proceed successively in regions where bundle sheath cells, bundle sheath extension and rib parenchyma cells, and armed mesophyll parenchyma cells will develop.

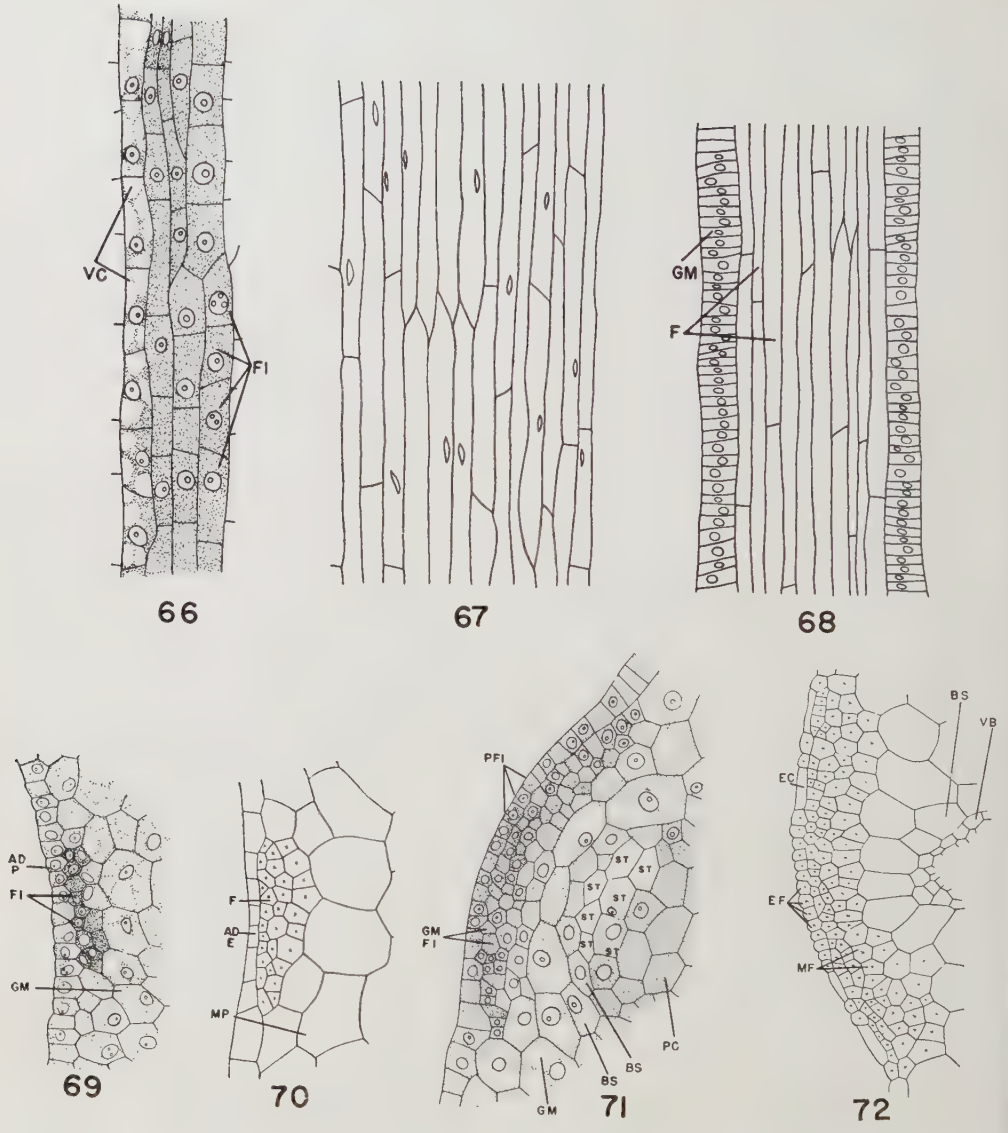
Armed mesophyll parenchyma cells are the last of the various cell types to differentiate in the lamina. However, the basipetal wave of development of lacunae, diaphragms and fibers continues in the sheath while armed mesophyll parenchyma cells are differentiating in the lamina. Thus, the late development of the armed mesophyll does not negate the concept of basipetal maturation of the leaf.

During plastochrons 4 and 5 ground parenchyma cells between vascular bundles in the lamina begin to enlarge and become highly vacuolate. Subsequently, chloroplasts appear in the cells, and the walls become " infolded " (Figs. 58, 59). The actual development of this apparent " infolding " has not yet been studied. In longisections one observes the elongated intercellular spaces being formed between the radial walls of the armed mesophyll cells. In the mature lamina a ladder-like pattern of narrow armed mesophyll parenchyma cells is evident (Figs. 9, 10). There is no armed parenchyma in the mature sheath. Rather, mesophyll cells located between the lacunae of the sheath wings simply enlarge and become highly vacuolate (Fig. 56). Ultimately they appear devoid of protoplasts.

DEVELOPMENT OF FIBERS — The structure and distribution of mature fibers in the rice plant have been described by deHaan (1911), Hector (1936), Juliano & Aldama (1937) and Duong-Huu-Thoi (1941). In the rice shoot, fibers occur in the stem nodes and internodes, the leaf sheath and lamina. In the leaf they form U-shaped or bar-shaped strands adjacent to the vascular bundles (abaxial in the sheath and abaxial and adaxial in the lamina), isolated strands near the adaxial epidermis of the sheath, and irregular bands in the epidermis of lamina and sheath.

The initiation of fibers occurs in the rice leaf as follows. Cells adjacent to the vascular bundles in the abaxial and adaxial layers of the ground meristem of the lamina begin to divide anticlinally and periclinally during plastochron 3. In the midrib portion of the lamina only the abaxial layer is involved. These divisions are initiated in the center of the lamina and subsequently proceed toward the margins of the wings. During plastochron 4 they proceed from the lamina into the sheath. Some fiber initials in the sheath are depicted in Figs. 69, 71. The initials increase in width and may divide longitudinally, obliquely, and transversely (Fig. 66). The continuity of the original files of cells in the ground meristem in these regions is thus interrupted. Subsequently the fiber initials elongate appreciably. Adjacent protoderm cells on the adaxial side also elongate and differentiate into fibers.

The elongation of fibers occurs during the period of most rapid elongation of the leaf, toward the end of plastochron 3 in the lamina, continuing through plastochrons 5 and 6 in the sheath. Judged by the numbers of fibers seen in transverse sections before and after elongation, the location of nucleus and cytoplasm in elongating fibers, and the relative positions of fiber apices during elongation, no evidence for apical intrusive growth could be found. The process of fiber elongation



FIGS. 66-72 — (*ADE*, adaxial epidermis; *ADP*, adaxial protoderm; *BS*, bundle sheath; *EC*, cells of adaxial epidermis which are not fibers; *EF*, epidermal fibers; *F*, elongating fibers in Fig. 68, mature fibers as marked by " x " symbols in Fig. 70; *FI*, fiber initials; *GM*, ground meristem cells; *GM FI*, ground meristem fiber initials; *MF*, mesophyll fibers; *MP*, mesophyll parenchyma cells; *PC*, procambium; *PFI*, abaxial protoderm fiber initials; *ST*, young sieve-tube members; *VB*, vascular bundle; *VC*, vacuolate cells of rib meristem in sheath midrib). Figs. 66-68. Stages in ontogeny of leaf fibers. Fig. 66. Ground meristem from median l.s. of sheath midrib near adaxial protoderm. × 790. Fig. 67. Strand of young fibers in l.s. of sheath. × 700. Fig. 68. Paradermal section of portion of sheath wing just below epidermis, showing young elongating fibers between two layers of plate meristem cells of future mesophyll. × 790. Figs. 69-72. Location of fiber initials and fibers in relation to vascular bundles and mesophyll parenchyma in the sheath. Fig. 69. Group of fiber initials adjacent to *adaxial* protoderm and surrounded by highly vacuolate cells of ground meristem. × 530. Fig. 70. Group of mature fibers adjacent to *adaxial* epidermis and surrounded by large mesophyll parenchyma cells. × 475. Fig. 71. Group of fiber initials derived from *abaxial* protoderm and from the ground meristem. × 1050. Fig. 72. Group of mature fibers of abaxial epidermis and in mesophyll in contact with bundle sheath cells of a vascular bundle. × 475.

appears to occur by a symplastic type of growth. Supporting evidence includes (a) the central positions of lens-shaped nuclei in young fiber cells (Fig. 67) and the fairly uniform distribution of cytoplasm in elongating fibers; and (b) no increase in the number of fibers in cross sections during their elongation.

Fully differentiated fibers are illustrated in Figs. 70, 72. These fibers have lignified secondary walls which first become evident during plastochrons 5 and 6. Some circular to irregularly rounded depressions, which appear to be simple pits, occur in the secondary walls. The length of mature fibers in the rice leaf varies from 110 to 299μ. The shortest fibers occur in the ligule region; these short fibers are characteristically attenuated at both ends. In contrast, lamina and sheath fibers typically occur in superposed series, the members being separated from each other by oblique or transverse walls.

Development of Stomata — The initiation of stomata occurs basipetally in the protoderm of an elongating leaf; this constitutes further evidence for the basipetal maturation of the leaf. In the development of stomata the following events take place. Alternate cells in a longitudinal file of cells in the protoderm remain comparatively short and densely cytoplasmic in contrast to the other protodermal cells, which elongate and become highly vacuolate (Fig. 73). These short cells are the guard cell mother cells. They appear in longitudinal series alternating with similar series of protodermal cells that do not give rise to stomata. In each large protodermal cell, laterally adjacent to each guard cell mother cell, a small subsidiary cell is cut off (Figs. 74, 75). First, a subsidiary cell is produced on one and then on the other side of the guard cell mother cell (Figs. 75, 76). Proximity of nuclei of a newly-formed subsidiary cell and the cell adjacent to the guard cell mother cell and occasional division figures constitute fairly clear evidence for the above interpretation (Fig. 74). The two narrow subsidiary cells on either side of the large guard cell mother cell widen considerably, and concomitantly, the guard cell mother cell divides anticlinally. While the two products of this division, the young guard cells, enlarge, a dark lenticular region (probably pectic material) becomes manifest in the center of the wall separating them (Fig. 77). During subsequent development the dark material disappears, and an intercellular space becomes evident. Concurrently, the guard cells become greatly elongated and dumbbell-shaped (Figs. 9, 10, 78). The nucleus in each of these cells is elongated. The development of stomata in rice leaves agrees with that reported for other grasses (cf. Esau, 1953).

Development of Cork and Silica Cells — As in other grasses, rice contains cork and silica cells in the epidermis. They may occur in pairs irregularly arranged in association with the long cells of the epidermis, or the silica cells alone may alternate with the short epidermal cells in long series arranged in parallel rows (Juliano & Aldama, 1937; Duong-Huu-Thoi, 1941). A study of the ontogeny of these cells was made in connection with the investigation of leaf elongation.

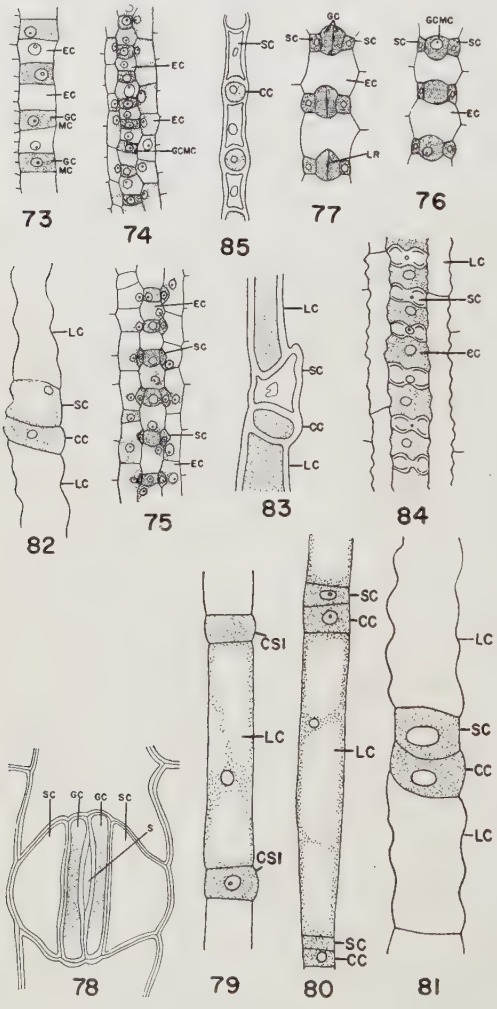
Early in the development of the sheath epidermis, alternate cells in the longitudinally oriented series can be observed to remain shorter and more densely cytoplasmic than cells above or below them (Fig. 79). These short cells divide anticlinally (Fig. 80). The products of the division enlarge (Figs. 81, 82) and gradually change in form. The upper cell of a pair — the silica cell — becomes filled with silica and loses its protoplast whilst the lower cell — the cork cell — retains its protoplast (Fig. 83). In the sheath, these cells are intercalated between long cells of the epidermis (Fig. 85). In the lamina, silica cells occur as single idioblasts alternating with short epidermal cells (Fig. 84). The silica cells are easily identified, for they reflect light in such a way that they appear nearly hyaline and exceedingly bright under the microscope. As with the development of guard and subsidiary cells, fibers, lacunae and diaphragms, the cork and silica cells are first initiated at the tip of the leaf and appear at successively lower levels as the leaf elongates.

Development of Trichomes — In the rice plant trichomes are present along the surface of the stem, lamina, sheath, glume,

lemma and palea; along the margins of the auricles; and at the tips of the ligule and leaf primordia. The morphology of fully differentiated trichomes of the rice plant has been described in detail by Juliano & Aldama (1937) and Duong-Huu-Thoi (1941).

A study of trichome development in the leaf epidermis was undertaken in connection with the studies on leaf maturation. As with the other types of epidermal cells the differentiation of these structures occurs in a basipetal direction. During initiation of a trichome a protodermal cell begins to enlarge more rapidly than the ones immediately above or below it in the same series (Fig. 86). Vacuolation becomes increasingly apparent, and the outer tangential wall grows outward (Figs. 87, 88); thus, the cell forms an elongated protuberance (Fig. 89). The nucleus and cytoplasm are retained even in mature trichomes (Figs. 90-92).

Development of Bulliform Cells — Bulliform cells in the rice leaf are restricted to the adaxial epidermis of the lamina. They occur in rows parallel with and between the longitudinally oriented vascular bundles and, in transections, form fan-like patterns at regular intervals in the



Figs. 73-85.

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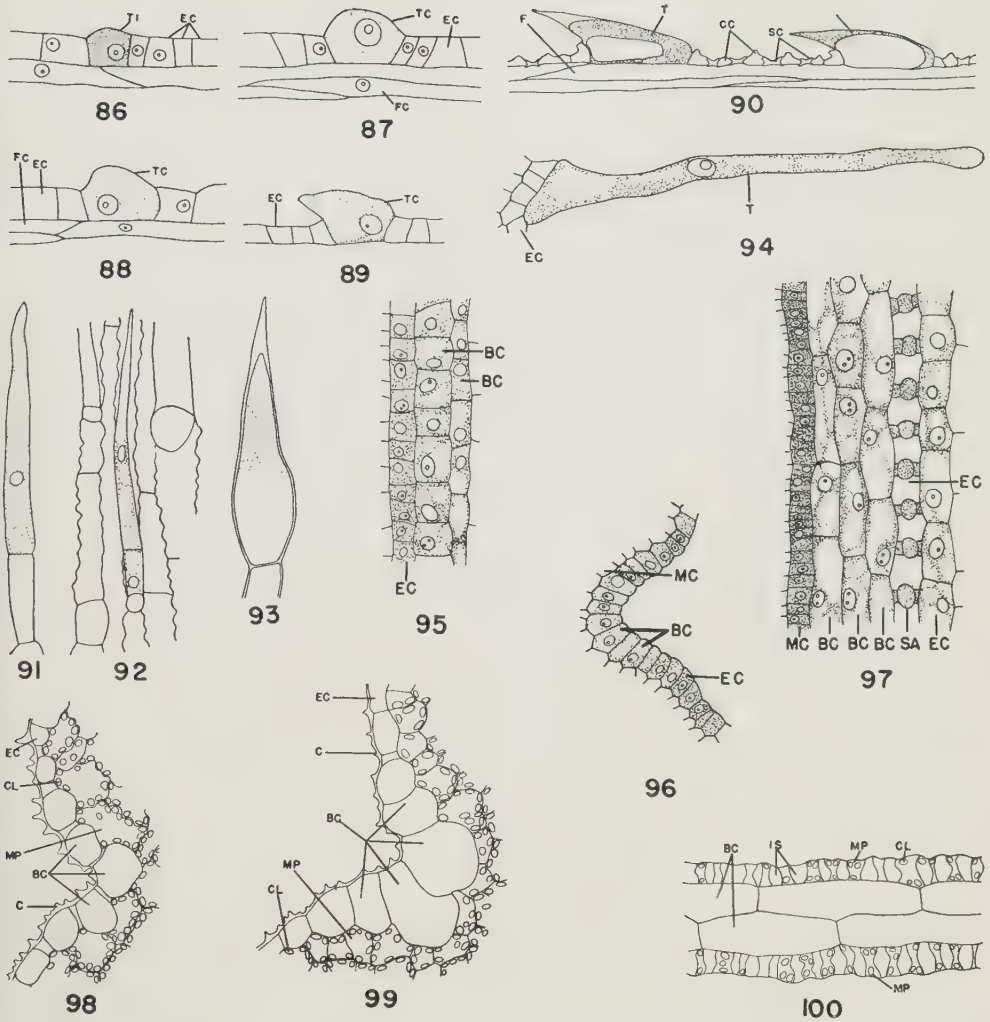
Figs. 73-85. Figs. 73-78 — (*EC*, epidermal cell; *GC*, guard cells; *GCMC*, guard cell mother cell; *LR*, lenticular region of pectic material, the stomatal pore precursor; *S*, stomatal aperture; *SC*, subsidiary cell). Stages in the ontogeny of stomata. Fig. 73. View of guard cell mother cells (stippled) alternating with epidermal cells. × 750. Fig. 74. First stage in formation of subsidiary cells. × 450. Fig. 75. Stage where many subsidiary cells have been formed. × 450. Fig. 76. Series of epidermal cells alternating with guard cell mother cells and associated subsidiary cells. Considerable cell enlargement and spacial readjustment have occurred since subsidiary cells were formed. × 750. Fig. 77. Young stomatal apparati, each composed of two subsidiary cells, guard cells and a lenticular region (stomatal pore precursor). × 750. Fig. 78. Fully developed stomatal apparatus. × 750. Figs. 79-85 — (*CC*, cork cell; *CSI*, initial of cork and silica cells; *EC*, epidermal cell; *LC*, long epidermal cell; *SC*, silica cell). Stages in ontogeny of silica, cork and long cells of the leaf epidermis. Fig. 79. Paradermal view of series of young epidermal cells. Elongated vacuolate cell is future long epidermal cell and two short densely cytoplasmic cells are initials of cork and silica cells. × 1500. Figs. 80, 81. Paradermal views of recently formed cork and silica cells and long epidermal cells. × 1500. Fig. 82. Paradermal section of lamina epidermis, showing parts of two long epidermal cells, a young vacuolate silica cell and a densely cytoplasmic cork cell. × 1100. Fig. 83. Radial section of lamina epidermis through pair of mature cork and silica cells and parts of two long epidermal cells. × 1100. Fig. 84. Paradermal section of lamina epidermis, depicting several long epidermal cells with undulate walls and a series of mature silica cells alternating with densely cytoplasmic epidermal cells. × 490. Fig. 85. Radial section of sheath, illustrating spherical, densely cytoplasmic cork cells alternating with silica cells. × 940.



FIGS. 86-100 — (*BC*, bulliform cells; *C*, cuticle; *CC*, cork cells; *CL*, Chloroplasts; *EC*, epidermal cells; *F*, fibers; *FC*, fiber cells; *IS*, intercellular spaces; *MC*, mesophyll cells; *MP*, mesophyll parenchyma; *SA*, stomatal apparatus; *SC*, silica cells; *T*, *TC*, trichome; *TI*, trichome initial). Figs. 86-90. Ontogeny of the unicellular epidermal trichome of the lamina. Fig. 86. Trichome initial and adjacent epidermal cells. × 1610. Figs. 87-89. Successively later stages in trichome development. Each. × 3470. Fig. 90. Two mature trichomes, adjacent epidermal cells and fibers. × 610. Figs. 91-94. Mature unicellular and two-celled trichomes. Fig. 91. Two-celled trichome with nucleated upper cell. × 1620. Fig. 92. Spacial relationship of two-celled trichome to lamina epidermis. × 840. Fig. 93. Distal cell of another type of two-celled epidermal trichome; upper cell has large basal vacuole. × 1620. Fig. 94. Young unicellular auricle trichome and adjacent epidermal cells of auricle at left. × 760. Figs. 95-100. Stages in ontogeny of bulliform and associated cells in lamina epidermis. Figs. 95-97. × 620; Figs. 98-100. × 440. Fig. 95. Paradermal section, illustrating a series of epidermal cells and two series of vacuolating bulliform cells. Fig. 96. T.s. depicting epidermal cells and enlarging bulliform cells. Fig. 97. Paradermal section, showing longitudinal series of cells that include, left to right, one series of mesophyll cells, three series of young bulliform cells, one series of epidermal cells alternating with stomatal apparati, and a series of epidermal cells. Figs. 98, 99. T.s. showing cuticle, mature bulliform cells, epidermal cells adjacent to bulliform cells, and mesophyll parenchyma (Fig. 98 represents an earlier stage than Fig. 99). Fig. 100. Paradermal section through grooved part of a lamina, depicting two series of mature bulliform cells and two series of mesophyll parenchyma cells with alternating intercellular spaces.

adaxial epidermis (Figs. 58, 59, 98, 99). The mature cells appear to be devoid of cytoplasm and nuclei and possess weak non-lignified walls. Usually bulliform cells are considered to be alive at maturity (cf. Esau, 1953).

Initiation and differentiation of bulliform cells proceeds basipetally in the lamina. Initiation begins during plastochron 4. Young bulliform cells can be differentiated from adjacent protodermal cells by their relatively early vacuolation and enlargement (Figs. 95-97). With continued differentiation these cells enlarge greatly, especially longitudinally (Fig. 97), and become increasingly more vacuolate. Mature bulliform cells and adjacent epidermal cells are shown in Figs. 9, 58, 59, 98, 99, 100.

Discussion

Leaf primordia initiation follows somewhat varied patterns in the monocotyledons. In the rice plant, leaf initiation is indicated by an increase in frequency of anticlinal divisions in the tunica, a phenomenon which also occurs in *Elodea* (Herrig, 1915; Stant, 1952). Both tunica and corpus contribute to the formation of leaf primordia in rice, a developmental relationship also encountered in *Iris* and *Vanilla* (Rüdiger, 1939) and in *Sorghum bicolor* and *Oplismenus imbecillis* var. *variegatus* (Thielke, 1951). In the region of initiation of the leaf primordium the cells comprising the outermost tunica layer in the rice shoot apex divide both anticlinally and periclinally, contributing to the formation of both protoderm and ground meristem in the primordium. This is also true of many other monocotyledons.

Various types of so-called meristems have been recognized in the morphogenesis of the rice leaf. The sequence and duration of activity of these meristems determine the stages in the development of the leaf. Initially, the primary meristematic tissues, protoderm, ground meristem and procambium, become delimited in the leaf primordium. The protoderm continues its cytokinetic activity through several plastochrons, ultimately differentiating into the epidermis. The procambium may be said to differentiate from the ground meristem. Different parts of the ground meristem show distinct patterns of growth. An adaxial meristem, first delimited in the leaf primordium, plays a major role in the formation of midrib and central regions of lamina and sheath, respectively. Marginal meristems, first active in the wing margins of the leaf primordium, are responsible for growth of wing margins of leaf primordia and young laminae and sheaths. A rib meristem, first organized in the lamina midrib, functions in elongation of lamina and sheath. A plate meristem functions in extension of lamina wings. Finally, superposed on these several meristems is the intercalary meristem, which during leaf elongation becomes progressively localized in the leaf base. It is obvious that the ultimate form of a grass leaf, such as that of rice, is largely dependent upon the time and duration of activity of the various meristems which function during leaf development.

According to Roth (1949), in the simplest cases of leaf development in monocotyledons (e.g. Amaryllidaceae, Liliaceae, Gramineae), the leaf primordium is hood-shaped and develops into a young leaf as a bifacial structure (abaxial and adaxial sides distinct from leaf base to leaf apex throughout development) and does not have ventral meristem activity (adaxial meristem). In contrast to Roth I observed an adaxial meristem in the rice leaf. Admittedly, this adaxial meristem is not as dominant a meristem and as active as that of certain other monocotyledons; however, it does play a major role in development of the midrib region of lamina and sheath in young rice leaves.

Prantl (1883) characterizes the pattern of growth in grass and conifer leaves as *basiplastic*, which implies that the leaf primordium as a unit passes from an entirely meristematic structure to an organ in which growth, in terms of cell division and enlargement, progresses from the apex toward the base. The direction of differentiation of the various meristems and cell types, such as, bulliform cells, trichomes, guard cells and subsidiary cells, armed and stellate parenchyma cells, and fibers, clearly suggests a basiplastic

pattern of growth and differentiation in the rice leaf.

Summary

Leaf primordia are initiated in the tunica, which contributes to both protoderm and ground meristem. The leaf primordium, at first a localized protuberance, develops around the shoot apex as a crescent-shaped, centrifugally growing organ. Later it grows upward, becoming hood-shaped. Marginal growth then initiates wing development. A generalized meristematic activity and apical growth account for elongation of the primordium. With initiation of ligule and auricles the leaf primordium becomes a young leaf composed of lamina and sheath. Various meristems are concerned with continued elongation and expansion of the leaf: rib, plate, adaxial, marginal, and intercalary meristems. The direction of cellular differentiation and maturation in the leaf is basipetal, as evidenced by the activity of these meristems and the direction of differentiation of lacunae and diaphragms, fibers, and various epidermal cell types. This activity ceases by the end of plastochron 6.

This paper is the second part of a revised and condensed thesis submitted to the Graduate Division of the University of California in partial fulfilment of the requirements for the degree of Doctor of Philosophy (*see* Kaufman, 1959).

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4. In many manuscripts sent to the editors, footnotes, bibliographies, descriptions of figures, etc., are typed single spaced. Since such material requires as much editorial marking as the rest, authors are urged to use double spacing throughout.

5. Literature citations are to be typed double spaced like rest of the manuscript. Give complete citation: author, year, title, name of journal, volume number and inclusive pages. In abbreviating names of journals follow " World List of Scientific Periodicals ", or the " U.S. Department of Agriculture list of abbreviations ", or the list given in Biological Abstracts. The words ' Indian ' and ' Indiana ' should not be abbreviated. A sample citation is given below:

STOKEY, A. G. 1948. The gametophyte of *Actiniopteris australis*. J. Indian bot. Soc. **27** : 40-49.

6. The patterns of headings should be as simple as possible and not more than three classes are to be used. Headings of the first class should be underscored with a bold wavy line, of the second with two parallel straight lines and of the third with a single straight line.

7. Drawings should be made with pen and undiluted India ink for reproduction by the zinc etching process. Group as many drawings as possible and mount them close together on heavy white cardboard for reduction to the width of single column (6 cm) or to the full width of the page (13 cm). A part or all of the length of the page (20 cm) may be utilized but it would be helpful if some space is left at the bottom to permit insertion of legend below the figures. It is not possible to accept loose figures or figures so mounted as to leave large unused spaces between them. Author's name and figure number must be written on the back of each mount. Do not affix legends to the figures but type them double spaced on a separate sheet. Photographic prints should be glossy with strong contrasts. Line drawings will be preferred, however, both because of the lower cost of reproduction and their relative clearness. When submitting manuscript, give the original magnifications of the illustrations. These will be revised later in accordance with the reduction to which the figures are subjected in reproduction. Alternatively give a dimensional scale in microns or mm for each figure or for each group of figures differing in magnification.

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9. Place all acknowledgements at the end of the paper just after ' Summary ' and before ' Literature Cited '.

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